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Fat Distribution in Cysticercoids of the Cestode *Hymenolepis diminuta**

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Relatively few studies have been published on the distribution of fats in cestodes. A recent report by Hedrick (1958) deals with fat distribution in adults of *Hymenolepis diminuta* and *Raillietina cesticillus* but does not include observations on the larval stages. It was therefore thought desirable to investigate fat distribution in fully developed, normal cysticercoids of *Hymenolepis diminuta*. The results of these observations, using standard histochemical methods, are presented here.

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MATERIALS AND METHODS

Cysticercoids of *Hymenolepis diminuta* were raised in confused flour beetles, *Tribolium confusum,* maintained on enriched flour at 30°C. Cysticercoids were dissected from beetles in saline solution and treated as follows. One group was fixed and embedded in paraffin according to the technique of McManus (1946) as given by Bullock (1949); paraffin sections were then stained with Sudan Black or with Sudan IV. Another group of cysticercoids was embedded in gelatin and frozen sections were stained with Sudan Black or with Sudan IV as described by Baker (1944) and Bullock (1949). Frozen sections from gelatin-embedded material were also used in the Smith-Dietrich test for the demonstration of phospholipids and cerebrosides, and in the Schultz cholesterol test. For procedures in both tests see Lillie (1954). All sections were cut at 10 microns.

For the purpose of orientation, a few comments on the structure of the cysticercoid are desirable. The thin outermost layer is referred to as the external membrane. The tissue internal to the membrane consists of numerous fine fibers (Fig. 2) which originate from a narrow layer of cells not apparent in the preparations shown here. This fibrous layer and the narrow cell layer are referred to as the peripheral layer of tissue. Beneath this is a region of relatively coarse cells (Figs. 1, 2) which appear to be parenchyma cells. The compact and narrow layer which completely surrounds the scolex is the wall of the cysticercoid cavity. Between the wall and the parenchyma cells is a dense fibrous layer which does not stain (Fig. 2) and will not be referred to subsequently.

Studies on the cytology of the cysticercoid of *H. diminuta* are now in progress and will be published elsewhere.

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RESULTS

SUDAN BLACK: Sections prepared from paraffin-embedded material (Fig. 4) show a heavier staining reaction than do gelatin-embedded sections. Intense stain deposits are present in the scolex and in the wall of the cysticercoid cavity. Within the cavity and anterior to the scolex one frequently finds several large, extracellular fat globules. The peripheral tissue layer and the narrow space beneath the external membrane appear dark blue and solid black respectively. In some specimens, the cysticercoid tail also stains heavily. The tissue between the wall of the cavity and the peripheral layer does not stain at all.

While sections of gelatin-embedded material (Fig. 2) show a stain distribution similar to that seen in paraffin sections, staining is much less intense in the scolex and in the peripheral layer. The cuticle of the scolex, the borders of the suckers and the wall of the cysticercoid cavity stain a solid dark blue or black, as does the space beneath the external membrane. Stain deposits in the tail are usually confined to the space beneath the external membrane. The extracellular fat bodies anterior to the scolex appear dark in some sections and pale in others. A large number of very small, darkly staining droplets may be present in the extracellular spaces between the scolex and the tissue immediately surrounding it. Small dark granules are frequently present throughout the muscular region of the suckers; these granules appear to be intracellular. Some specimens contain large numbers of deeply staining extracellular bodies inside the cavity anterior to the scolex. The relatively large parenchyma cells adjacent to the peripheral layer (Fig. 2) stain darkly at what appear to be the cell boundaries.

In general, different specimens vary considerably in the intensity of the staining reaction.

SUDAN IV: Paraffin-embedded sections (Fig. 3) stain a brownish-red in the scolex, in the wall of the cysticercoid cavity and in the tail. The stain is most intense in the wall of the cavity. The space beneath the external membrane stains lightly or not at all. No droplets or granules were seen within the cysticercoid cavity or elsewhere.

Gelatin-embedded sections (Fig. 1) appear similar to paraffin sections except that the external membrane stains more heavily in the former. Numerous darkly staining droplets are present in the cysticercoid cavity, particularly anterior to the scolex. Very little stain is deposited in the tail, and none in any of the tissues between the wall of the cavity and the peripheral layer. The latter may contain a few very small dark granules.

SMITH-DIETRICH TEST: Gelatin-embedded sections treated by this procedure show the following: A bluish-grey color in the space beneath the external membrane, in the wall of the cysticercoid cavity, and in the cuticular layer of the scolex. The staining reaction is most intense in the wall of the cysticercoid cavity. Under high magnification one observes numerous small, bluish-grey granules, scattered among the fibers of the peripheral tissue layer. There are no black staining areas.

SCHULTZ CHOLESTEROL TEST: A pale bluish-green color is present throughout the region of the scolex and the wall of the cysticercoid cavity.

INTERPRETATION OF RESULTS: It is evident that a considerable amount of fat is present consistently in certain areas of the cysticercoid. From comparison of sections stained with Sudan Black with those stained with

Sudan IV it may be assumed that most of the fat in the scolex, in the cavity and in the wall of the cavity is true fat. However, the relatively intense reaction obtained in the peripheral layer with Sudan Black indicates the presence of at least one other substance, perhaps phospholipid

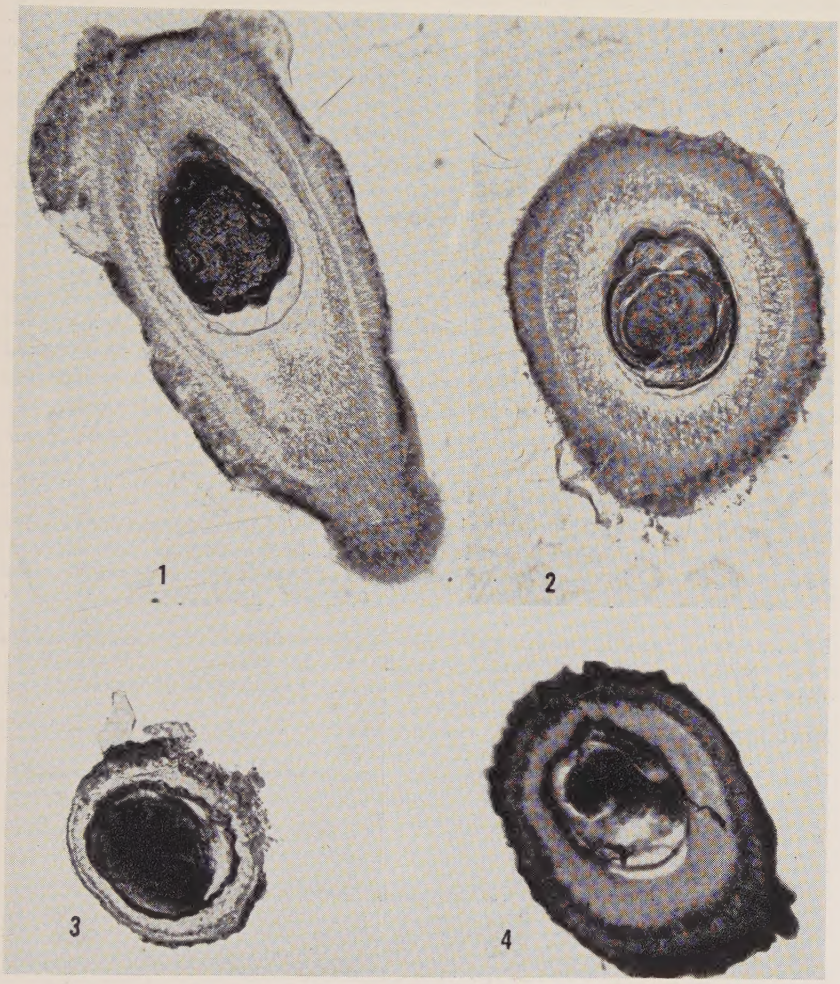

Fig. 1. Gelatin-embedded section of *Hymenolepis diminuta* cysticercoid stained with Sudan IV. Note heavy stain deposit around scolex and beneath peripheral membrane; large extracellular fat bodies are present within the cysticercoid cavity.

Fig. 2. Gelatin-embedded section stained with Sudan Black. Staining reaction similar to that in Figure 1 except that peripheral layer stains more intensely.

Fig. 3. Paraffin-embedded cross-section stained with Sudan IV. Stain is most intense in wall of cysticercoid cavity and beneath peripheral membrane.

Fig. 4. Paraffin-embedded section stained with Sudan Black. Note relatively intense staining reaction of the peripheral tissue layer, the parenchyma cells and the scolex.

(See Bullock, 1949, p. 206). Results of the Smith-Dietrich test however do not confirm the presence of phospholipid but suggest the possibility of a cerebroside in the wall of the cavity and in the cuticle of the scolex. In addition, a positive reaction for cholesterol was obtained in the wall of the cysticercoid cavity and in the scolex. Consequently, there is good evidence that in addition to true fats, the cysticercoid contains other related substances.

The true fats possibly represent storage products. This may also be true for polysaccharide deposits known to be present in the cysticercoids (Heyneman and Voge, 1957). Information on the function of these substances might be gained by starvation of the infected intermediate host and subsequent examination of the cysticercoids (Reid, 1942). The relation of fats in the wall of the cysticercoid cavity to excystment of the cysticercoid in the definitive host remains to be determined.

SUMMARY

The presence of fatty substances in the cysticercoid of *Hymenolepis diminuta* has been demonstrated by histochemical methods. True fats appear to be concentrated in the peripheral tissue layer, the wall of the cysticercoid cavity, the cavity proper, and the scolex. Positive reactions for a cerebroside and for cholesterol were obtained in different areas of the cysticercoid.

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The Origin of the Gelatinous Matrix in *Meloidogyne*

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There are few references in the literature concerning the origin of the gelatinous matrix in *Meloidogyne* spp. Nagakura (1930) considered the matrix to be a uterine secretion. Results of the present study indicate that at least part of the gelatinous matrix is produced by six rectal glands which deposit their secretions through the anal opening. The nuclei of these glands were described and illustrated by Nagakura but he referred to them as being nuclei of the vaginal muscles. Elsea (1951) concluded that these "Giant Nuclei" were associated with the excretory system and made no reference to the possibility that they were in reality the nuclei of the matrix glands.

METHODS AND MATERIALS

Observations were made on lactophenol prepared perineal sections with attached body contents, glycerine prepared totomounts of adults, juvenile females, second stage larvae, living specimens and stained serial cross sections. Four species of *Melodogyne* were studied: *Meloidogyne incognita acrita* Chitwood, 1949, *M. javanica javanica* (Treub, 1885) Chitwood, 1949, *M. hapla* Chitwood, 1949 and *M. arenaria thamesi* Chitwood, 1952.

Living specimens were used in order to see the matrix actually being deposited. These specimens were prepared by placing a galled piece of root in water and dissecting away the root tissue from the posterior end of the mature female. Galls and females prepared in this manner were then placed on a slide in water and covered with a coverslip supported on three heavy glass rods about the same diameter as the galls. A small gall on a fine root was found to be the most satisfactory, because the posterior portion of the female is easily exposed by dissection. Water level in the preparation was maintained by renewing from a pipette at variable intervals.

Specimens to be microtomed were processed through a standard paraffin embedding technique. Specimens were killed by gentle heating over an alcohol lamp and then fixed in F.A.A. (distilled water, 120 parts; 95% alcohol, 60 parts; formalin, 18 parts; glacial acetic acid, 3 parts). Specimens were left in the fixative for a minimum of 24 hours.

Dehydration was accomplished in a well slide by passing the specimens very slowly through the series of alcohol and xylene. Each change of alcohol was carried out by initially substituting single drops of the desired percentage until approximately half of the solution had been replaced. All of the solution was then drawn off and replaced with the desired concentration. This same technique was applied to the alcohol xylene substitutions.

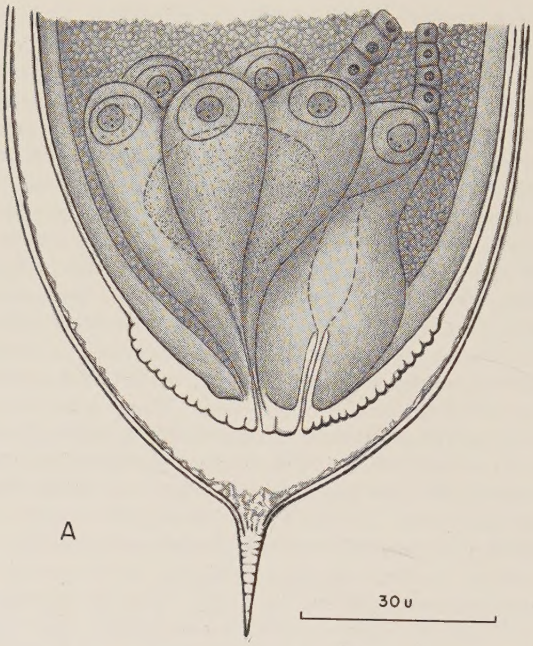
In order to effect infiltration paraffin chips were added to the final xylene change and the specimens left in an infiltration chamber for 24 hours. The chamber consisted of a depression slide with a watch glass inverted over the well.

Chitwood and Chitwood's 1930 procedure for embedding was followed. Sections were cut at 6 microns. A standard regressive iron hematoxylin staining procedure as outlined by Pantin (1948) was employed. Sections were stained with Baker and Jordan's (1953) modification of Heidenhain's iron hematoxylin. A 2½% Ferric Alum solution was used as a mordant and a saturated solution of Picric acid in 95% ethyl alcohol as a destain.

DISCUSSION

There are six rectal matrix glands in *Meloidogyne* spp.: two subdorsal, two lateral and two subventral. The presence of these glands was first suspected while studying perineal sections of *Meloidogyne* spp. with the body contents still attached. The gland ducts leading to the rectum were observed but seldom could the entire gland be seen. Thick perineal sections allow the observation of the glands and their nuclei.

In totomounts of mature and juvenile females of *Meloidogyne* mounted in glycerine or Lacto-phenol the glands and their nuclei are easily visible (Fig. 1, A & B). The six glands at their widest point almost touch laterally and thus are almost continuous around the body just under the hypodermis. The gland nuclei are the largest nuclei found in the body of mature female *Meloidogyne*. The nuclei are 25-32 microns through their longest axis and the



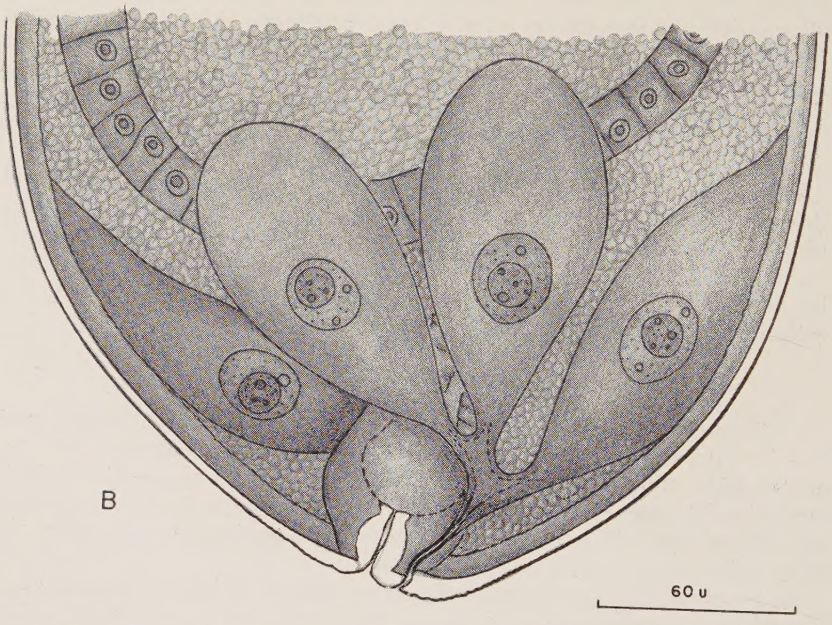


Fig. A. *Meloidogyne incognita acrita*, juvenile female within spike tailed cuticle, showing position of matrix glands (totomount in glycerine)

Fig. B. *Meloidogyne incognita acrita*, mature female, illustrating four of the six matrix glands (totomount in glycerine)

nucleoli are 11-15 microns in diameter.

Matrix glands are not readily discernible in totomount specimens of the second stage larvae, however, careful examination shows them to be present. They are readily visible in all later stages and even in the second stage larvae at the time it begins to swell. In juvenile females (females still encased by the second stage cuticle) of *Meloidogyne* the six glands occupy approximately 1/6th of the body length and are concentrated more posteriorly than in the fully developed female (Fig. 1, A). When the female first forms inside the spike tailed cuticle, the gland nuclei are 10 microns through their longest axis and 4-4.5 microns in diameter. As the females swell and proceed to full maturation the glands and their nuclei also enlarge. The glands prior to egg production occupy approximately ¼th of the total body length.

The rectal lumen is large and easily visible when females have just completed the final molt (Fig. 1, A). As the female matures the rectal cavity becomes smaller (Fig. 1, B) and more difficult to see.

The general shape of the individual glands is pyriform, the gland ducts being drawn out considerably. The nuclei are located in the anterior half of the glands.

The gelatinous matrix emanates from the anal opening and is exuded as a thread. The newly formed matrix observed under oil emersion is seen to have a fibrous appearance as reported by Chitwood (1938), this is

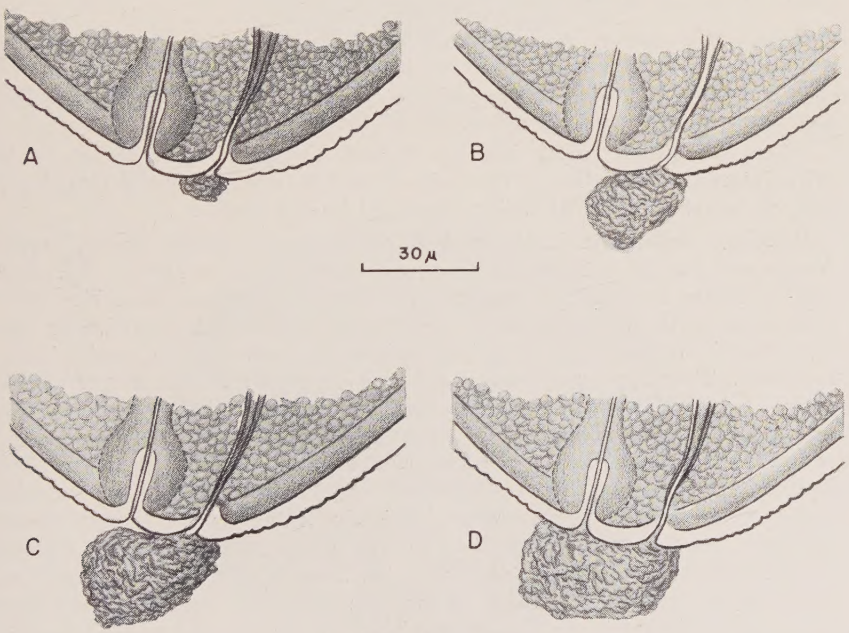


Fig. A. *Meloidogyne arenaria thamesi.* Beginning of matrix deposition from anal opening.

Fig. B. Matrix after two hours.

Fig. C. Matrix after three hours.

Fig. D. Matrix after four hours.

a result of the threadlike deposition. That the matrix is at least in part a product of the glands in *Meloidogyne* is established by the observations made on living material in the process of depositing the matrix and on fixed, stained serial sections.

The serial illustrations portraying matrix deposition, over a period of four hours, were made with a camera lucida from a living specimen (Fig. 2, A, B, C, D). The illustrations were made at hourly intervals beginning with the first sign of matrix deposition and concluding at the time the source of its production became obscured. This living female still embedded in the root was observed for a period of six hours.

Prior and during matrix production, the female was observed to pulsate the rectum by contraction of the dorsal depressor ani muscle. The rate of pulsation approximated one contraction every ten seconds under the conditions of the observations (Temperature, 70°F.). The depressor ani muscle continued these contractions, at a constant rate, throughout the period of observation.

The gelatinous matrix first appears as a small fibrous mass or globule, which continues to flow from the anus at a steady but slow rate (Fig. 2, A). After three hours the matrix had just about obscured the vulva (Fig. 2, C). However, the matrix at this time was still distinct from the vulva and was never seen to exude from the uterus or vulva. Four hours after the gelatinous matrix deposition began the vulva-anal region was completely covered and observations were halted (Fig. 2, D).

Hematoxylin stained cross sections show the glands, at the level of the nuclei and anterior to them to be evenly distributed around the body and closely appressed to the hypodermis (Fig. 3, A). At their deepest point the glands extend into the body one-fourth of the body diameter.

Where the glands empty into the rectum, the position of the ducts is shifted dorsally in relation to the body, so that the two subventral glands are furthest apart (Fig. 3, B), being separated by the uterus.

Histologic differences in the nucleoli of the matrix glands of the young female and the fully mature female were apparent (Fig. 1, A & B). The nucleoli in the immature female are granulated and stain a dark blue with iron hematoxylin. As the female approaches maturation and during the time she is depositing a matrix, decided changes in the nucleoli are apparent. They appear, at maturity, to be vacuolated as well as granulated (Fig. 3, A). These apparent vacuoles or globules in the nucleoli are not artifacts and were verified in variously fixed and prepared materials: lactophenol preparations, glycerine mounts and paraffin sections. The stained cross sections show that there is a correlation between these vacuoles and the chemistry of the nucleolus, as is illustrated by the change in the nucleoli's reaction to hematoxylin. In the adult female about to deposit a matrix or in the process of doing so, the nucleoli stain an oily green and the vacuoles or globules have a yellow hue; this is in contrast to the even blue reaction in the immature female. These changes in the nucleoli suggest that possibly the glands are stimulated to begin matrix production by a secretion from the nucleoli.

Throughout the matrix glands sinus canals are visible (Fig. 3, A), these unite in each gland to form a single duct that enters into the anterior extremity of the rectum (Fig. 3, B). It appears that the initial part of the ducts, at the junction of the glands and rectum, are cuticularly lined. The

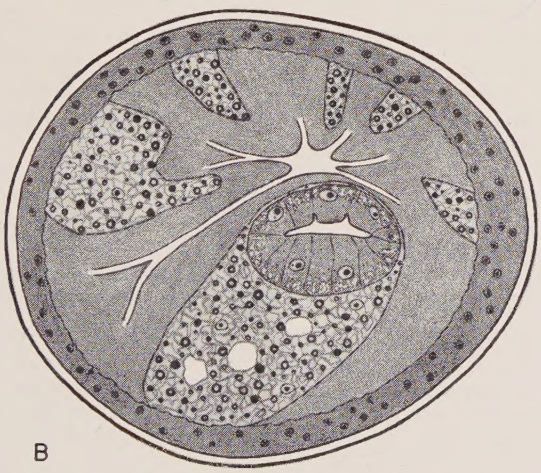


Fig. A. *Meloidogyne incognita acrita.* Cross section at level of matrix gland nuclei, sinus canals in the matrix glands are indicated by clear areas.

Fig. B. *Meloidagyne incognita acrita.* Cross section showing ducts of matrix glands entering into the rectum.

remainder of the glands have a rather homogenous consistency and little or no change in appearance is apparent with age, with the exception of increased number of sinus canals in older females. The glands react to hematoxylin by staining an even light blue. The sinus canals have no color in stained sections, however, in old females some material, probably matrix is seen to adhere to the walls of these canals.

In *Meloidogyne* the hypodermis is an unusually thick syncytial layer surrounding the entire inner surface of the cuticle. The tissue here considered as hypodermis was considered a degenerate muscle layer by Elsea (1951). The hypodermal nuclei are small and numerous (Fig. 3, A & B). A discrete somatic muscle layer was not visible.

The intestine in the mature female loses all cellular identity and the intestinal nuclei are scattered throughout the stored food granules (Fig. 3, A). The intestine is a syncytium without any anatomical evidence of a defined lumen. No connection could be established between the intestinal syncytium and the rectum. In the stained serial sections the rectum can be seen to terminate at the junction of the gland ducts. The only visible openings are those where the rectal matrix glands enter the rectum (Fig. 3, B).

Rectal glands have not been previously reported to be present in the Tylenchoidea (Chitwood and Chitwood, 1950). The matrix glands of *Meloidogyne* may be considered to be rectal glands with a specialized function. Rectal glands occur in other orders of the Secernentea, normally three in females and six in males. It is possible that the six glands in females of *Meloidogyne* are the result of duplication. The occurrence of an intestinal syncytium has been reported by Chitwood (1951) and it is now established that in adult females of *Meloidogyne* there is no connection between the intestinal syncytium and the rectum.

SUMMARY

The gelatinous matrix of *Meloidogyne* is described as a product of six glands which enter into the anterior extremity of the rectum. These matrix producing glands are described from totomounts, lactophenol or glycerine preparations, living specimens and hematoxylin stained serial cross sections.

In *Meloidogyne* the intestine is a syncytium without discrete cells and has no connection with the rectum.

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Seasonal fluctuations in larval trematode infections in *Stagnicola emarginata angulata* from Phragmites Flats on Douglas Lake

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Stagnicola emarginata angulata (Sowerby), the common beach snail on Douglas Lake, has been examined for larval trematodes ever since 1914. Repeated examinations have been made over the years from accessible places on the lake shore where this snail is abundant. However, there are very few collections made in different years in which the incidence of larval trematode infection can be directly compared. In 1937, Cort, McMullen and Brackett (1937) compared the trematode infections in collections of this snail from four different areas on Douglas and Burt Lakes. From one of these areas, Phragmites Flats, two collections of snails were examined in the summer of 1936, on July 6 and August 26. Twenty years later collections of the same snail species made at different times during the summer were examined from exactly the same area. A comparison of the larval trematode infections in these five collections is summarized in Table I.

The series of examinations for larval trematodes for 1956 and 1957 shows a rather low incidence (15.0 per cent) in juveniles collected on August 8, 1956. The incidence of positives in this same generation of snails after living over the winter (July 1, 1957) was still lower (12.4 per cent). However, during the month of July the snails of this area acquired a lot of new infections so that the incidence in the collection of July 29, 1957 had increased to 60.0 per cent. This was due almost entirely to increase in infections with *Diplostomum flexicaudum* although infections with *Plagiorchis muris* also greatly increased. Of the 37 unidentified positives (immature infections) 30 were strigeoids (probably mostly *D. flexicaudum*) and 7 were stylets (probably *P. muris*). There were only three double infections in this collection, one of an immature strigeoid plus *P. muris,* the second an unidentified tailless cercaria plus *D. flexicaudum* and the third the tailless cercaria plus an immature strigeoid. This absence of double infections was partly due to the fact that *D. flexicaudum* and *P. muris* which were the most common species in this collection are rarely found together in double infections (cf. Cort et al., 1937). The great increases in the incidence of *D. flexicaudum* and *P. muris* over the month of July is the only very striking thing in this seasonal comparison. When we consider that the herring gull is a common host for both these trematodes and that *P. muris* is found also in the sand-piper the reason for this rapid increase is evident.

When the 1936 examinations are compared with the 1956-1957 series it can be seen immediately that there have been very great changes in the larval trematode infections harbored by this species of snail over that period of more than 20 years. In the first place the incidence of infection is considerably higher in the July 6, 1936 collection than it is in the July 1, 1957 collection (38.4 per cent compared with 12.4 per cent). Also in the second collection examined in 1936, which was made almost a month later than that in 1957, the incidence of larval trematode infection is somewhat greater (77.4 per cent as compared with 60 per cent). However, the greatest difference

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between these two collections is the much larger variety of trematode species found in 1936, some of which had a fairly high incidence. The incidence of infection with the larval stages of *D. flexicaudum* is considerably less in 1936 (20.4 per cent as compared with 32.0 per cent). Very striking is the high incidence of the larval strigeoid which was identified as *Cotylurus communis* in the 1936 collections. Olivier and Cort (1942) demonstrated experimentally that the cercaria that Van Haitsma assigned to *Cotylurus communis* could not possibly belong to this species. Therefore this cercaria should be called *C. emarginatae* Cort, 1917. The high incidence of this species in the 1936 collections (14.7 and 11.9 per cent respectively) is very surprising since only 1 infection was found in the later series of examinations. Another difference between the two sets of examinations is the higher incidence of the bird schistosome, *Trichobilharzia stagnicolae,* in the 1936 collections. These collections also show a much larger variety of larval trematode species and a high incidence of *P. proximus,* which is not reported at all in the 1956-1957 series. It should be noted, however, that the cercaria of *P. proximus* is difficult to distinguish from that of *P. muris,* but the presence of McMullen in the group examining the 1936 collections made it much easier to separate these two plagiorchioids since he was working for his doctor's thesis on that group.

Perhaps the most striking difference between the 1936 collections and those made in 1956-1957 was the very large number of multiple infections in the snails of the earlier collections, especially that made on August 26, 1936. In the 383 snails in that collection positive for larval trematode infection there were 42 double and two triple infections. The combinations in these multiple infections and the numbers of each are shown in Table 2.

It has been realized for several years that the larval trematode infections in the beach snails of the Douglas Lake region have been greatly reduced over the years that investigations have been made in the area. This reduction has been both in the number of different species present, and in the incidence of infection. It has been suggested that the number of vertebrates, especially

TABLE 1. Larval trematodes in collections of *S. emarginata angulata* from Phragmites Flats on the north shore of Douglas Lake

Dates of collections	7/6, 1936	8/26, 1936	8/8, 1956*	7/1, 1957	7/29, 1957
Total number of snails	753	495	326	201	309
Number of negative snails	464	112	277	176	123
No. positive for larval trematodes	289	383	49	25	186
Number of unidentified positives	30	12	12	10	37
Number of identified positives	259	371	37	15	149
Cer. *Diplostomum flexicaudum*	50	101	32	8	100
Cer. *Cotylurus communis***	111	58			
Cer. laruei	1	10	1		
Cer. yogena	12	18			10
Cer. dohema	1				
Cer. *Cotylurus flabelliformis*	1	1			
Cer. emarginatae					1
Cer. *Trichobilharzia stagnicolae*	22	36		2	3
Cer. *Schistosomatium douthitti*		1			
Cer. *Plagiorchis muris*	27	44	3	5	31
Cer. *Echinoparyphium recurvatum*	1	3	1		
Cer. *Notocotylus urbanensis*	1	1			
Cer. *Eustomos chelydrae*	3				
Cer. *Plagiorchis proximus*	29	98			

*Large juveniles; the other 4 collections are of adults

**This cercaria should be designated as *Cer. emarginatae* Cort, 1917.

water birds living on the beaches and visiting them, has been greatly reduced over the years, because of the greatly increased number of summer cottages. This is probably the reason for the great reduction in trematode infections in the beach snails.

TABLE 2. Combinations of species of larval trematodes found in multiple infections in a collection of *S. emarginata angulata* made from Phragmites Flats on Douglas Lake on August 26, 1936.

Double infections	Number of multiple infections
C. Diplostomum flexicaudum + *C. emarginatae****	11
C. D. flexicaudum + *C. laruei*	2
C. D. flexicaudum + *C. yogena*	2
C. emarginatae + *C. yogena*	1
C. D. flexicaudum + *C. T. stagnicolae*	6
C. emarginatae + *C. T. stagnicolae*	1
C. laruei + *C. T. stagnicolae*	1
C. yogena + *C. T. stagnicolae*	2
C. yogena + *C. P. muris*	1
C. laruei + *C. P. proximus*	1
C. T. stagnicolae + *C. P. muris*	3
C. T. stagnicolae + *C. P. proximus*	7
C. P. muris + *C. P. proximus*	2
Triple infections	
C. D. flexicaudum + *C. emarginatae* + *C. yogena*	1
C. yogena + *C. T. stagnicolae* + *C. P. muris*	1

***This form was incorrectly considered to be the cercaria of *Cotylurus communis*.

SUMMARY

A collection of juveniles of *S. emarginata angulata* was made from Phragmites Flats, an area at the north end of Douglas Lake, Michigan on August 8, 1956. This was followed the next summer by two collections of adults of the same generation made on July 1 and July 29, 1957. The incidence of infection with larval trematodes was low in the first two of these collections, but was quite high in the third. Only infections of *D. flexicaudum* and *P. muris* were present in any numbers. Collections of the same snail species made in 1936 from exactly the same area had been examined for their larval trematode infections. In them there was a much higher incidence of larval trematode infection with a considerably larger number of species. The collection made on August 26, 1936 had 77.4 per cent larval trematode infections with 11 different species, while that made on July 29, 1957 had 60 per cent infection and 5 different species. The 1936 collection also had a large number of multiple infections, 42 double and two triple. The July 29, 1957 collection had only three double infections. It is suggested that the reduction in trematode infections is due to a reduction in the number of vertebrate hosts, especially water birds, that visit the beaches because of the greatly increased number of summer cottages.

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Nematode Parasites and Associates of the California Five-spined Engraver, *Ips confusus* (Lec.)

Calvin L. Massey*

The California five-spined engraver is one of the most serious insect pests of young ponderosa and pinyon pine in southwestern United States. In 1957 it killed a major portion of the pinyon on 800,000 acres of woodland type in Arizona and New Mexico. Outbreaks were especially serious in the northern half of New Mexico. As with other species of *Ips,* outbreaks are sporadic. Struble and Hall (1955) considered it as one of the most destructive killers of young pine forests in California and southern Oregon.

Since research by the writer (Massey 1954) had revealed that the internal nematode parasites belonging to the genus *Aphelenchulus* and *Sphaerularia* were important factors in the ecology of the Engelmann spruce beetle in Colorado, studies were initiated in 1957 on the nematode parasites and associates of *Ips confusus* to determine their effect in the rise and fall of epidemics of this insect.

Internal nematode parasites were obtained by dissecting live adult beetles, pupae and larvae of the California five-spined engraver. Associated nematodes were washed from beetle galleries. In 1958 beetles were examined from each of the four generations to determine if a variation existed in numbers of adults infested per generation.

Nematode Endoparasites of *Ips confusus*

Three endoparasites were recovered from adults, pupae and larvae of the beetle. Two species were from the body cavity and belong to the genera *Aphelenchulus* and *Parasitaphelenchus.* Larvae of *Rhabditis obtusa* Fuchs were recovered from the gut of the adult and larval stages of the insect. Descriptions of the species of *Aphelenchulus* and *Parasitaphelenchus* follow.

Aphelenchulus elongatus, n. sp.

Eggs: Deposited before segmentation, .021 mm. × .068 mm., laid in body cavity of infested beetles.

Larvae: Length .58 mm. Width .016 mm. Cuticle very finely striated, lip region rounded, set off by a very slight depression. Fig. 1 D. Spear slender, knobbed, .014 mm. in length, comparable in length to body width. Esophagus a narrow tube narrowing even more as it passes through a prominent nerve ring. Excretory pore slightly posterior to the nerve ring. Genital primordia apparent. Anal opening not discernible. Terminus moderately obtuse. Fig. 1 E.

Immature females: Length .78 mm. Width .042 mm. Cuticle very finely striate, becoming almost annulate in the region of the head. Body tapering only slightly towards head, beginning to constrict ventrally and bend slightly dorsally in the region of the vulva. A series of cells with very large nuclei at the anterior 1/3 of the body. Lip region narrowly rounded. Spear .014 mm. in length, finely knobbed. Esophagus a narrow tube; lumen of the esophagus traceable posterior to the nerve ring. Nerve ring prominent. Genital primordia extending halfway to the anterior end. Vulva a prominent broad transverse slit; terminus to vulva .07 mm. Anal opening not discernible. Terminus narrowly rounded.

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The research reported was completed at the Station's Forest Insect Laboratory at Albuquerque, New Mexico.

MATURE PARASITIC FEMALES: Length 4.25-5.45 mm. Width .12-.14 mm. Cuticle moderately thick, transversely striated, the striations coarser in the region of the head. Body tapering slightly towards the head, bent dorsally in the region of the vulva. Body tends to coil when nema is killed by heat. Lip region as in Figure 1 A, B. Spear .014 mm. in length. Lumen of the esophagus visible for only a short distance from the spear. Ovary outstretched, ½ to ¾ the body length. Vulva a broad transverse slit. Anal opening not discernible. Tail broad, obtuse with distinct mucro. Length from terminus to vulva .36 to .45 mm. Fig. 1 C.

MALE: Unknown.

DIAGNOSIS: *Aphelenchulus* with elongate body dorsally bent in region of vulva. Allied to *A. grandicolli* Massey (1957) but much longer than that species. The lip region is more narrowly rounded and the distance from the terminus to the vulva is greater than in *A. grandicolli*.

TYPE LOCALITY: Bandelier National Monument, New Mexico.

TYPE HOST: *Ips confusus* (Lec.).

LIFE HISTORY NOTES: Several hundred larvae, pupae and adults of the California five-spined engraver have been examined for nematode parasites. Adult beetles were infested to a greater extent than larvae and pupae. Observations indicate that infection takes place through the oral cavity. Hundreds of eggs and larvae are present in the body fluids of the abdominal cavity of the host insect. First stage larvae evidently penetrate the wall of the gut and pass out of the insect with the fecal material. Infective stage larvae are found in numbers in the frass of the beetle galleries.

Several juvenile females may occupy the body cavity, ten being the maximum number found in one adult beetle. The adult female parasites, relatively

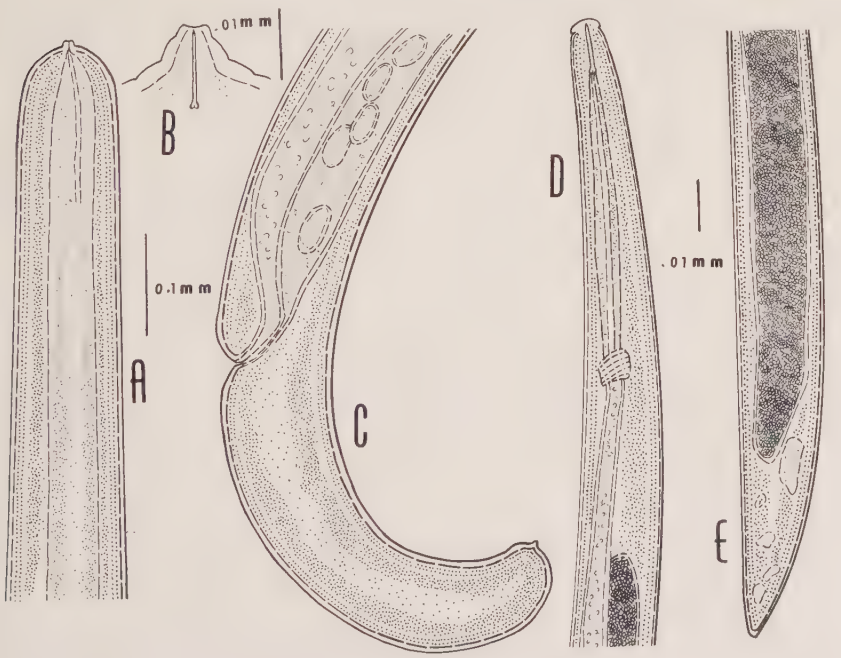

Figure 1. *Aphelenchulus elongatus*, n. sp. A. Head; B. Lip region and stylet; C. Tail; D. Head larva; E. Tail larva.

lethargic, entwine themselves around the malpighian tubules and reproductive organs, remaining in this attitude until egg laying is complete. Only the females occur in the body cavity. Males are unknown.

NUMBERS OF BEETLES PARASITIZED: In 1957, 615 beetles were examined from 17 trees; 41.3 percent were infested with *Aphelenchulus elongatus*. Equal numbers of males and females were infested. In 1958, 568 beetles were examined from 21 trees; 28.7 percent were infested. Approximately 10 percent more females than males were parasitized. In 1958 beetles were examined from four generations (Table 1). The number of beetles parasitized

Table 1. Parasitization by generation of *Ips confusus* by *A. elongatus*. Bandelier, New Mexico, 1958.

Generation	Percent Infested		
	Male	Female	Total
I	14.2	38.8	34.0
II	20.8	35.7	33.3
III	7.1	16.6	16.0
IV	28.5	12.9	17.3

in the first two generations was considerably higher than in the last two. It is thought that the parasites carried over in large numbers in the overwintering adult beetles and declined in numbers as the beetle infestation declined.

EFFECT ON HOST: *Aphelenchulus elongatus* is a true parasite. Controlled studies reveal that egg production and brood production of female *Ips confusus* infested with this nematode is greatly reduced.

Pairs of beetles were introduced into muslin sacks containing green pinyon bolts 3 to 5 inches in diameter and 15 to 18 inches in length. The beetles were reared in the bolts in a constant temperature and humidity cabinet (relative humidity 80 percent, 80° F.). One group was removed after two weeks to determine the number of eggs laid in that period. Another group was removed after four weeks to determine the numbers of progeny produced by each pair. As shown in Table 2 there was a 52 percent reduction in the

Table 2. Eggs laid in a two-week period by female *Ips confusus* infested with *Aphelenchulus elongatus*.

	No. of beetles	Eggs laid	Average per beetle
Infested	14	175	12.5
Non-infested	16	416	26.0
Dead	20	------	------

number of eggs laid by infested females. Maximum number of eggs laid by an infested female was 37; by an uninfested female, 62.

Table 3. Brood produced in 4 weeks by female *Ips confusus* infested with *Aphelenchulus elongatus*.

	No. of beetles	Brood produced	Average per beetle
Infested	15	365	24.3
Non-infested	27	1,563	57.9
Dead	25	------	------

There was a 58 percent reduction in the brood produced by infested females (Table 3). The maximum number of larvae, pupae and adults produced by infested females was 49 as compared with 108 by uninfested females.

These studies revealed that the beetle progeny was more likely to be infested with the nematode parasite when the female or both sexes were parasitized than when the male alone was infested. Forty-seven percent of the brood was infested when the adult female was parasitized; 53 percent, when both adults were infested; and 6.2 percent, when males were infested with *A. elongatus.*

There is a considerable difference in gallery lengths produced by infested individuals as compared with those that are uninfested. Beetles parasitized by the nema constructed egg galleries that averaged 4.5 inches in length as compared with egg galleries that averaged 7.1 inches in length for uninfested females.

Parasitaphelenchus sp.

A large proportion of the beetles collected during the course of the study were infested with a species of *Parasitaphelenchus,* which is probably undescribed. Very little is known of the parasite. Both Ruhm (1956) and Fuchs (1937) describe several species of this genus and have associated them with free living forms taken from the galleries of infested beetles.

Specimens of individuals belonging to the genus have been taken from all bark beetles examined over a period of years throughout the western United States. To date it has been impossible to make an accurate, authentic comparison of the immature parasites found in the body cavity with associated free living forms. Attempts to culture the parasitic forms have met with little success. Much work needs to be done before accurate descriptions of the species native to the United States can be made. Definitive internal characteristics are lacking in some forms.

The following description is made of the immature parasitic form taken from the body cavity of *Ips confusus.* Because of its immaturity it is not named. Length .63 mm. a = 40. b = 11. c = 24. Cuticle thin, finely striate. Body widest at the middle; narrowing sharply towards head, less sharply toward the tail. Lip region rounded with mucronatic projection as in Figure 2 A. Spear not visible, bulb of the esophagus nearly twice as long as wide. Nerve ring prominent, excretory pore slightly posterior to the nerve ring. Anal opening faintly discernible. Tail narrowly rounded with short sharp mucro as in Figure 2 B.

This species of *Parasitaphelenchus* was recovered from the body cavities of 41 percent of 775 beetles examined for the presence of *A. elongatus.* The nema has little or no effect on its host.

Rhabditus obtusa Fuchs, 1915

The immature forms of this nematode occur in the gut of *Ips confusus.* The adult forms occupy the egg and larval galleries of its host. Beetles infested with the larvae of the nema exhibit no ill effects from the infection. The heads and tails of the adults are shown in Figure 5 J, K, L. Thorne (1935) aptly describes the species.

NEMATODE ASSOCIATES OF *Ips confusus* IN PINYON PINE

Several nematodes were collected from the egg and larval galleries of the California five-spined engraver beetle. They were:

Diplogaster bandelieri, n. sp.

Aphelenchoides gallagheri, n. sp.

Cryptaphelenchus latus (Thorne, 1935), Ruhm 1956

Laimaphelenchus penardi (Steiner, 1914), Filipjev, Schuurmans-Stekhoven, 1941

Macrolaimus taurus Thorne, 1937
Rhabditis obtusa Fuchs, 1915
Descriptions of the two new species follow:

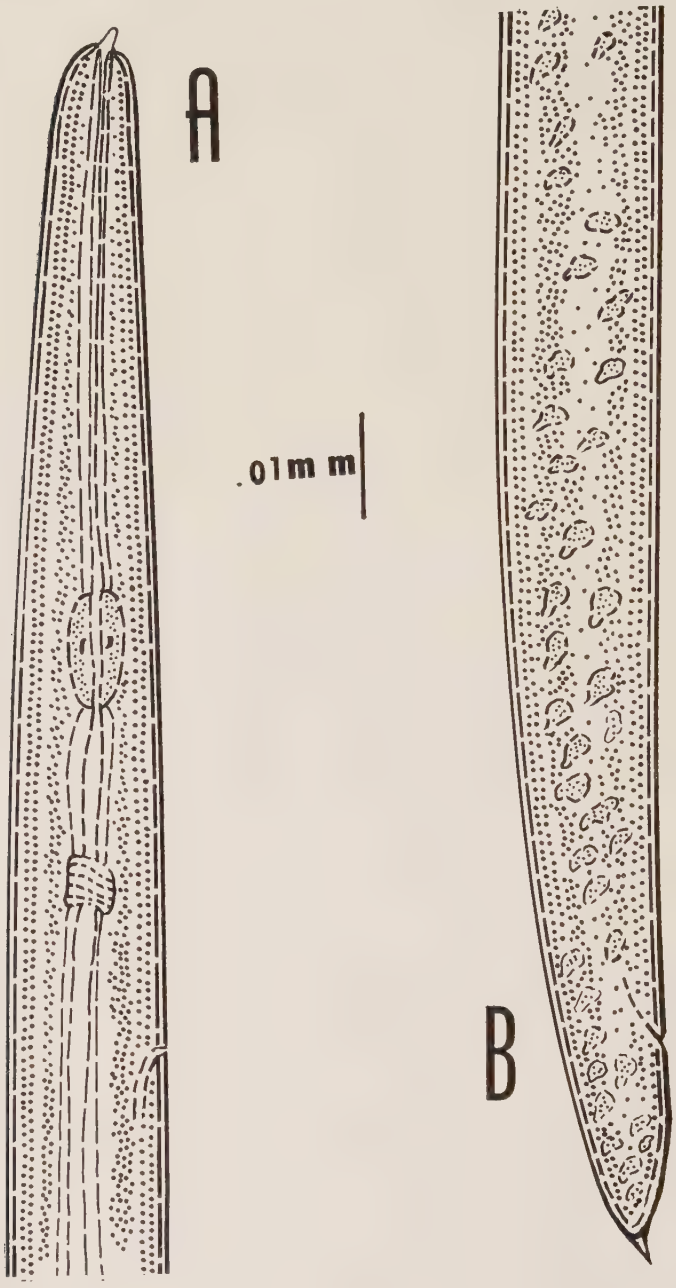

Figure 2. *Parasitaphelenchus* sp. (immature). A. Head; B. Tail.

Diplogaster bandelieri, n. sp.

FEMALE: 1.12 mm. a = 27. b = 5.3. c = 9. V = *26/57/21*.

MALE: .95 mm. a = 22. b = 5.6. c = 11. T = 65.

FEMALE: Cuticle very finely striate. Body moderately slender, widest near the middle, tapering only slightly toward the head. Lip region broadly rounded, almost square. Figure 3 A. Pharynx armed with 7 visable teeth, one of which is a large centrally located dorsal tooth. Nerve ring midway of the isthmus, excretory pore slightly anterior to the terminal bulb. Vulva a nearly transverse slit with protuberant labia. Amphidelphic, ovaries at times reflexed. Tail convex conoid, ending in an obtusely rounded terminus. Figure 3 C.

MALE: Similar in shape and conformation to female. Testes reaching nearly to the terminal bulb, at times reflexed. Ten pairs of caudal papillae. Spicula as in Figure 3 B. Arcuate cephalated. Gubernaculum thick proximally with a thin trough-like distal extension. Tail convex conoid, obtusely rounded. Figure 3 B.

DIAGNOSIS: *Diplogaster* with convex-conoid tail, broadly rounded lip region. Allied to *Diplogaster pinicola* Thorne but differs from that species in the more broadly rounded lip region and in its tail characteristics. The terminus of both sexes is more obtusely rounded than *D. pinicola*. The tail of the male not at all spicate.

TYPE LOCALITY: Bandelier National Monument, New Mexico.

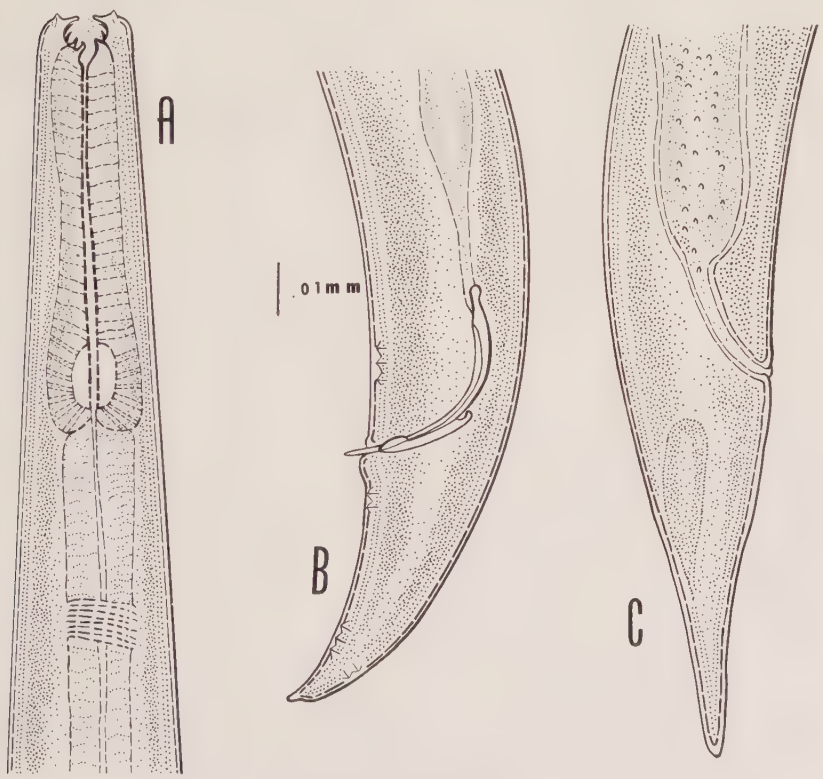

Figure 3. *Diplogaster bandelieri*, n. sp. A. Head; B. Male tail; C. Female tail.

Nearly all galleries of the California five-spined engraver were infested with specimens of this species. It is thought that the larvae of *D. bandelieri* is an ectoparasite of the adult beetles. Larvae of *D. pinicola* were collected extensively from beneath the wing covers of *Dendroctonus engelmanni* and reared to maturity in malt agar.

Aphelenchoides gallagheri, n. sp.

Female: 1.73 mm. a = 47. b = 23.5. c = 8. V = *76/90/5*.

Male: 1.0 mm. a = 39. b = 12.8. c = 5. T = 73.

Female: Cuticle marked with fine longitudinal striae. Body very slender, elongate. Lip region moderately rounded, set off by slight depression. Stylet long, slender, finely knobbed, .011 mm. long. Figure 4 A. Bulb of the esophagus oblong ovate, approximately twice as long as wide; 3 esophageal glands visible in lateral view. Nerve ring prominent, excretory pore only slightly posterior to nerve ring. Labia of vulva protuberant, the anterior more protruding than the posterior. Anterior branch of ovary extending ¾ of the distance to the esophogeal bulb, posterior branch extending nearly to anal opening. Tail broadly conical mucronate. Figure 4 C.

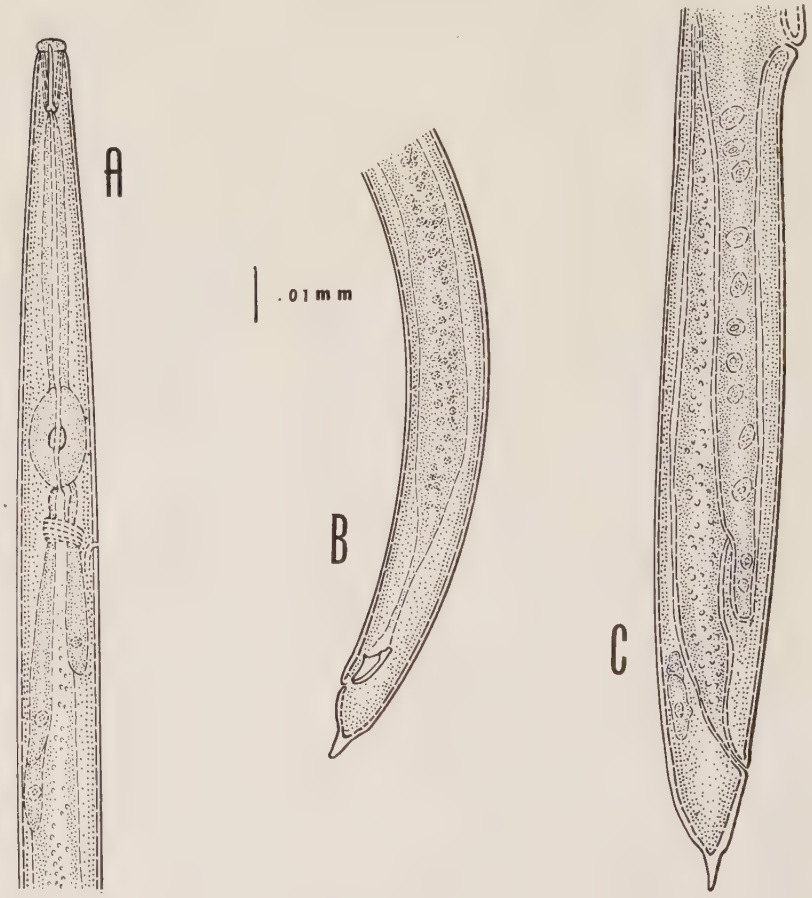

Figure 4. *Aphelenchoides gallagheri,* n. sp. A. Head; B. Male tail; C. Female tail.

MALE: Similar in shape and conformation to the female, shorter in length. Testes single, at times reflexed. Spicula small, angular in a lateral perspective. Tail broadly conoid, mucronate. Figure 4 B.

DIAGNOSIS: Slender elongate *Aphelenchoides* with mucronate terminus in both sexes. Closely related to *Aphelenchoides acroposthion* Steiner 1932 but differs in length, the tail shape of the female and the absence of discernible transverse striae.

TYPE LOCALITY: Bandelier National Monument, New Mexico.

Aphelenchoides gallagheri was collected frequently from the galleries of *Ips confusus*. It was also recovered from the galleries of *Ips ponderosae* in ponderosa pine.

The other species of nematodes, with the exception of *Macrolaimus taurus*, were collected only occasionally in association with the bettle. *M. taurus* was abundantly associated with the insect in all collections. *M. taurus, C. latus, L. penardi* and *R. obtusa* are illustrated in Figure 5 A through L.

SUMMARY

Three internal nematode parasites were collected from *Ips confusus*, *Aphelenchulus elongatus* n. sp., *Parasitaphelenchus* sp. and *Rhabditis obtusa* Fuchs. Studies carried on in 1958 revealed that the egg laying potential of females infested with *A. elongatus* was reduced by 52 percent, the brood producing potential by 58 percent. Beetles parasitized by *Parasitaphelenchus*

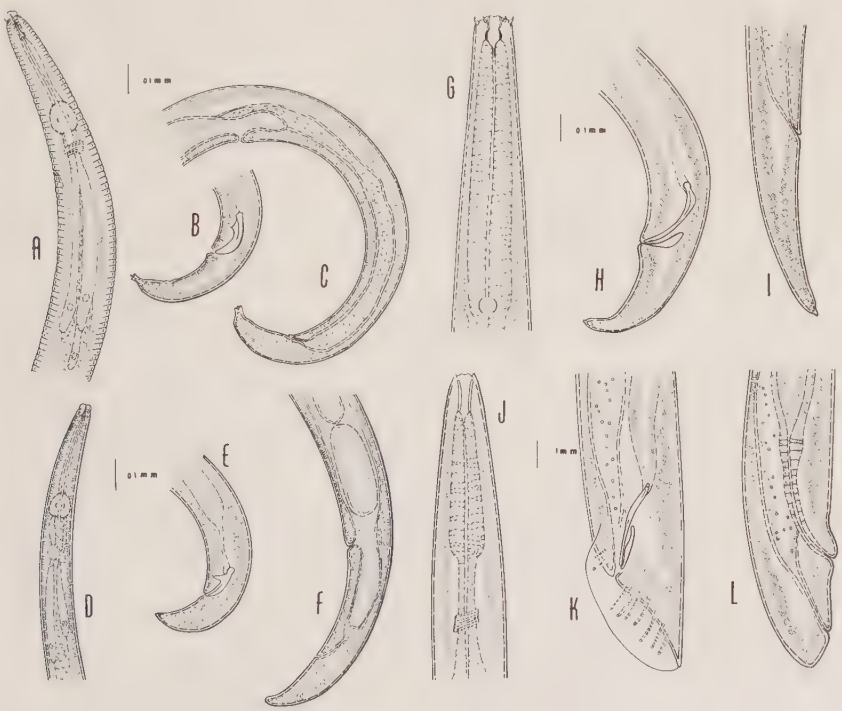

Figure 5. *Laimaphelenchus penardi* (Steiner), Filipjev, Schuurmans-Stekhoven. A. Head; B. Male tail; C. Female tail. *Cryptaphelenchus latus* (Thorne), Ruhm. D. Head; E. Male tail; F. Female tail. *Macrolaimus taurus* Thorne. G. Head; H. Male tail; I. Female tail. *Rhabditis obtusa* Fuchs. J. Head; K. Male tail; L. Female tail.

sp. were not affected by the infections. Nematodes collected from the egg and larval galleries were *Diplogaster bandelieri* n. sp., *Aphelenchoides gallagheri* n. sp., *Cryptaphelenchus latus* (Thorne), Ruhm, *Laimaphelenchus penardi* (Steiner), Filipjev, Schuurmans-Stekhoven, *Macrolaimus taurus* Thorne, and *Rhabditis obtusa* Fuchs.

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Two New Species of the Genus *Trichodorus* (Nematoda: Dorylaimoidea) from India*

M. RAFIQ SIDDIQI

Two new species of the genus *Trichodorus*, namely *T. mirzai* and *T. acaudatus* are described below. This appears to be the first record of *Trichodorus* spp. from Asia.

Trichodorus mirzai, n. sp.** (Fig. 1, A-I)

MEASUREMENTS: 2 males: Length = 0.518-0.52 mm.; a = 18-19.1; b = 4.5-5.1; c = 45-57.5; T = 62-63

3 females: Length = 0.492-0.609 mm.; a = 18.4-19.6; b = 4.6-6.3; c = sub-terminal; V = $^{15\text{-}25.6}$-54.1-54.5$^{14.6\text{-}21}$; onchiostyle = 31-34 microns.

MALE (HOLOTYPE): Length = 0.518 mm.; a = 18; b = 4.5; C = 45; T = 63.

Body cylindrical, tapering abruptly at the ends. When killed by gradual heat the worm becomes straight and the cuticle swells up considerably. Lip region about 7 microns in diameter. Amphids open at the base of the lateral lips through large apertures measuring 2.5 microns wide. Each amphid is continued posteriorly into a globular pouch of the same width containing bundles of hair-like sensillar elements. Onchiostyle or spear 29 microns in length the middle third of which is tripartite. Slightly anterior to the tripartite region it is surrounded by a guiding ring which has been shown by Allen (1957) to be a muscular collar where the lumen of the oesophagus empties into the pharynx. Longitudinal bands of muscle fibres which act as protrudors for the onchiostyle quite prominent.

Anteriorly, there are three large-sized ventromedian cervical papillae; the first from the anterior end of the body is situated at about one labial diameter

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**Named after Prof. M. B. Mirza, Head of the Zoology Department, Aligarh Muslim University.

and the second one spear length behind the base of the onchiostyle, the third is about 10 microns posterior to the second.

Oesophagus with an anterior slender tubular portion and a posterior bulb containing the oesophageal gland nuclei. Nerve ring slightly anterior to the

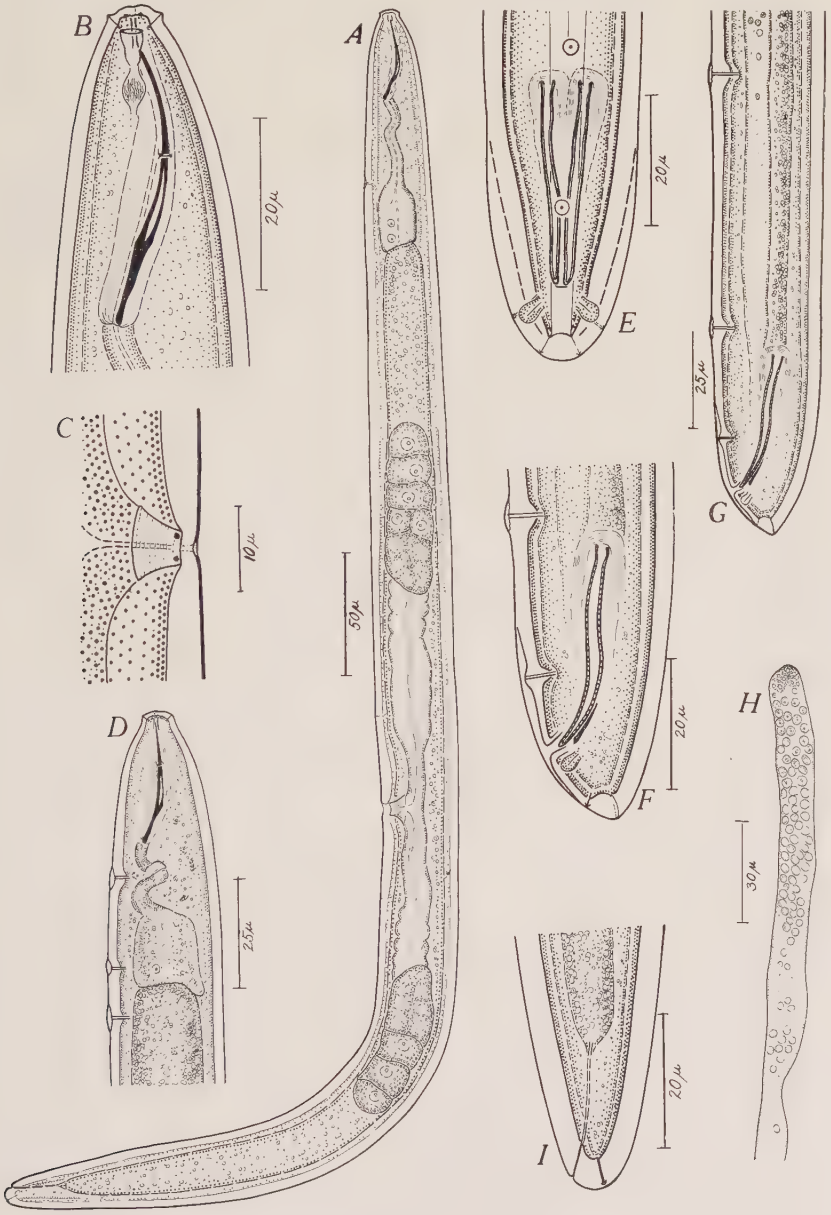

Figure 1. *Trichodorus mirzai.* A. Female. B. Anterior end of female. C. Vulva and vagina. D. Oesophageal region of male. E. Caudal end of male, ventral view. F. Caudal end of male, lateral view. G. Posterior end of male. H. Testis. I Caudal end of female.

middle of the anterior slender portion of oesophagus. Excretory pore could not be located, possibly as in the female. Oesophagus set off from the intestine. No cardia or oesophago-intestinal valve is seen. Intestinal cells packed with dense food granules.

Testis single, outstretched. Details of the formation of the sperms are very clear in the paratype male (Fig. 1, H). Supplements consist of three ventromedian papillae which extend to a distance approximately three spicula lengths from the anus. The first from the posterior extremity is located at a level slightly behind the middle of the spicula; the middle one slightly anterior to the level of the proximal ends of the spicula and the third lies at a distance about two-and-one-third times that between the first and the second.

Spicules paired, similar, and arcuate, measuring 32.5 microns in length. They are slightly cephalated and bear faint beaded markings throughout their length which give them a striated appearance. Gubernaculum is linear, approximately 11 microns long with a distal thickening. Circular protrudor muscles of the spicules are prominent.

A pair of large pedunculated ventro-submedian papillae is present just posterior to the anal opening. Another pair of post-anal papillae is located at about the middle of the distance from the first pair of the post-anal papillae to the paired terminal caudal pores. A weak, not very conspicuous, flap-like bursa begins from the level of the middle of the distance between first and second supplementary papillae and completely envelopes the tail.

Female (Allotype): Length = 0.609 mm.; a = 18.4; b = 6.3; c = subterminal; V = $^{25.6\text{-}54.1\text{-}20.8}$.

Body similar to that of male. Onchiostyle 33 microns long. Excretory pore located at the level of the distal end of the posterior bulbar portion of the oesophagus. Ventromedian cervical papillae absent. Oesophageal bulb set off from the intestine, contains about five gland nuclei.

Vulva a longitudinal, slit-like aperture, 2 microns in length. It leads into a short vagina provided with circular musculature. Cuticularized pieces surrounding vagina inconspicuous, dot-like (Fig. 1, C). From the vagina the two sets of reproductive organs diverge out in opposite directions. Uteri highly muscular, capable of accommodating more than one egg at a time. Ovaries well developed and reflexed approximately half-way back to vulva. From the cap-cell the oögonia arrange themselves in double rows and as they develop into large-sized oöcytes they come to lie in single file.

The posterior end of the body narrows rather abruptly to a rounded terminus. Rectum long and conspicuous, opening through a distinct, subterminal anus. Paired terminal caudal pores slightly dorsal in position.

Holotype: Male collected on April 12, 1957; slide deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

Allotype: Female collected on April 15, 1957 about roots of cabbage, *Brassica oleracea* L.; other data same as for holotype.

Paratypes: 1 male and 2 females; other data same as for holotype.

Type host: Collected around roots of cabbage, *Brassica oleracea* L.

Type locality: Aligarh (U. P.), India.

Diagnosis and relationship: *Trichodorus* with the above measurements and general description. It can be recognized by its small size of the body (0.492-0.609 mm.) and a comparatively small onchiostyle measuring 29-34 microns in length. In the male, by the presence and position of three ventromedian cervical papillae, three ventromedian supplementary organs, two pairs of post-anal papillae, a pair of terminal caudal pores and a thick-

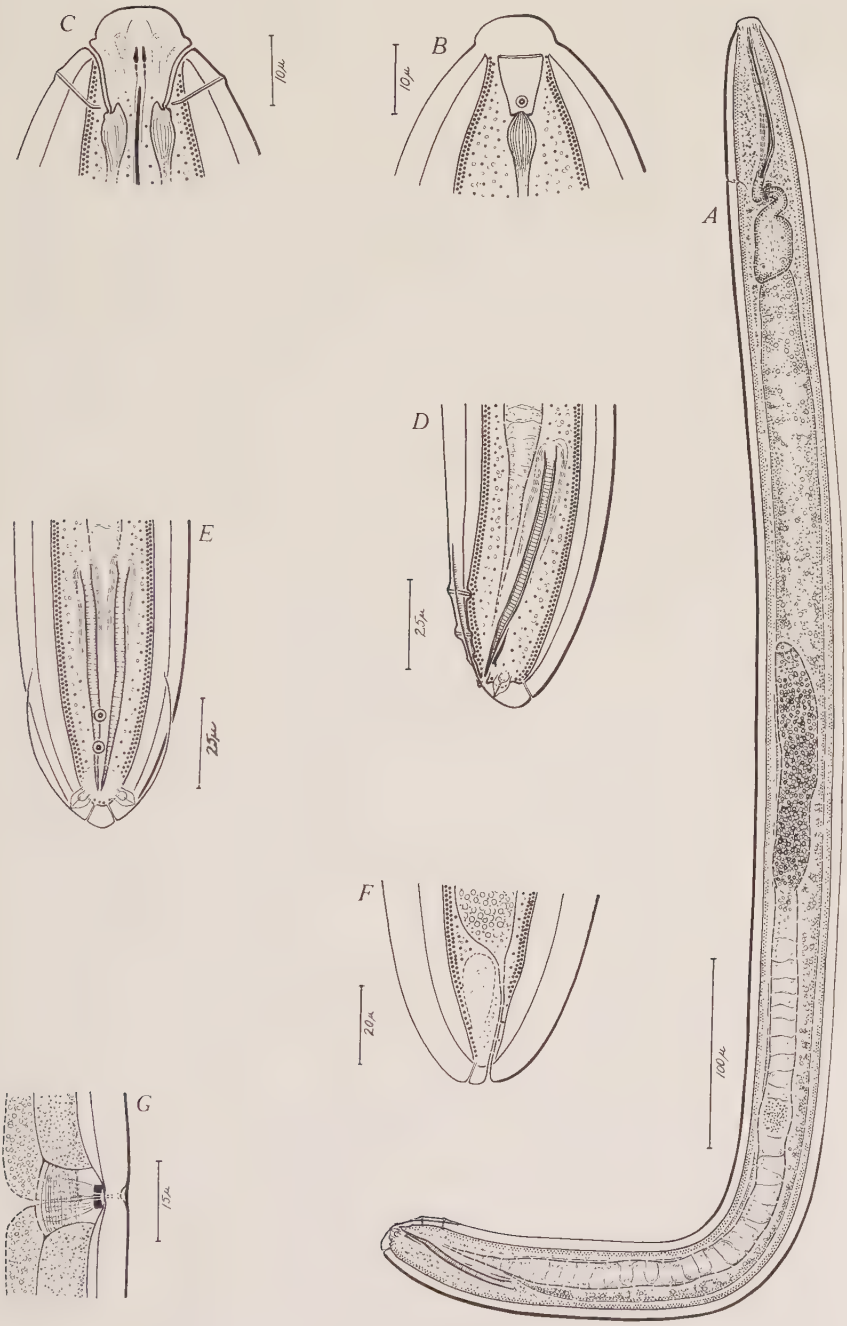

Figure 2. *Trichodorus acaudatus.* A. Male. B. Amphid and hypodermal pore of male, lateral view. C. Amphids and hypodermal pores of male, dorsal view. D. Posterior end of male, lateral view. E. Posterior end of male, ventral view. F. Posterior end of female, lateral view. G. Vulva and Vagina.

walled bursa. In the female, by the location of excretory pore, and presence of inconspicuous cuticularized pieces surrounding vagina, and by paired terminal caudal pores.

T. mirzai, n. sp. is closely related to *T. minor* Colbran, 1956 from which its female can be distinguished by having a well set-off oesophagus and oöcytes not arranged in a single file. Colbran (1956) examined several thousand specimens and could not find any males. In the present study, however, males were nearly as abundant as females. His description is based on females only and that too is very meagre. He has mentioned nothing about the excretory pore and the terminal caudal pores. From his illustration of an adult female, however, one can notice the presence of rod-like cuticularized pieces surrounding the vagina and the long ovaries which are reflexed two-thirds of the distance back to the vulva and with oöcytes in a single file.

In its small body-size and onchiostyle *T. mirzai* resembles *T. christiei* Allen, 1957 and *T. nanus* Allen, 1957 but its females can easily be distinguished by the more anteriorly located excretory pore and the presence of a pair of sub-terminal caudal pores. Other related species are *T. pachydermus* Seinhorst, 1954, and *T. primitivus* (de Man, 1880) from which it is distinctive because of the smaller size of the body, the onchiostyle and the spicules, the presence and position of the three ventromedian cervical papillae, and the spacing of the three supplementary organs in male.

Trichodorus acaudatus, n. sp. (Fig. 2, A-G)

Measurements: 3 males: Length = 0.75-0.847 mm.; a = 14.5-19.5; b = 5.7-6.1; c = terminal; T = 60-61; onchiostyle = 75-78 microns; spicules = 67-68 microns.

2 females: Length = 0.91-0.95 mm.; a = 15.8-18.2; b = 5.8-6; c = terminal; V = 52.4-54.7; onchiostyle = 82-83 microns.

Male (Holotype): Length = 0.847 mm.; a = 18; b = 6.1; c = terminal; T = 61.

Body covered with thick cuticle which swells up considerably and separates from the body when the animal is killed by gradual heat. Lip region hemispheroidal, set off from the body. Amphid apertures a pair of large slit-like openings situated at the base of the lateral lips. Each of these is 6 microns wide and leads into a laterally compressed, vase-shaped amphid at the base of which lies a globular pouch packed with hair-like sensillar elements. A pair of lateral hypodermal pores is situated at about the level of the base of the amphids (Fig. 2, B & C). Onchiostyle slender, 78 microns in length; muscular collar at the end of its anterior third; longitudinal muscle bundles for the onchiostyle distinct.

Oesophagus with a slender anterior and a bulbar posterior portion. Nerve ring slightly posterior to the base of the spear. Excretory pore opposite spear base. Three of the gland nuclei contained in the oesophageal bulb are distinct. A portion of the base of the oesophagus slightly extends over the anterior end of the intestine. Ventromedian cervical papillae absent. Intestinal wall filled with rounded granules.

Testis single, outstretched; cap-cell followed by few large-sized spermatogonia beyond which hundreds of spermatozoa pack the testis. Supplements in the form of two ventromedian papillae, confined to the bursal area. A thick-walled, finely striated bursa surrounds the anal opening. Spicules paired, slender, measuring 68 μ in length. They are almost straight except for a slight dorsal bend near the distal end. Transverse striations present on the spicules. Gubernaculum linear, 12 μ long bearing a dorsally directed

hook-like projection at its distal end. Anus almost terminal. A pair of large, pedunculated, ventro-submedian papillae is situated post-anally. Terminal caudal pores dorsally located.

FEMALE (ALLOTYPE). Length = 0.95 mm.; a = 15.8; b = 6; c = terminal; $V = {}^{14.5\text{-}}54.7^{\text{-}15}$.

Body similar to that of male. Amphids as described for holotype. Lateral hypodermal pores near lip region absent. Excretory pore located at a level slightly posterior to the base of the onchiostyle which is 83 microns long. Oesophageal bulb set off from the intestine.

Vulva a minute aperture without distinctive shape, leading into a transverse vagina whose inner lining is irregularly cuticularized. At its outer end it is guarded by cuticularized pieces forming a rim which is about 2 microns broad. To the inner face of this rim are attached muscles which run parallel to vagina. These are themselves surrounded by another set of muscles running parallel to body axis. Uteri long and muscular. Ovaries paired, reflexed less than half-way back to vulva.

Rectum 32 microns long, opening to the exterior through a terminal, transverse anus. Terminal pores slightly dorsal in position.

HOLOTYPE: Male collected from soil sample received in September, 1958;* slide deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

ALLOTYPE: Female collected from soil sample of sugarcane; other data same as for holotype.

PARATYPES: 2 males and 1 female; other data same as for holotype.

TYPE HOST: Isolated from soil sample of sugarcane (species not determined).

TYPE LOCALITY: Coimbatore (Madras State), South India.

DIAGNOSIS AND RELATIONSHIP: *Trichodorus* with the above measurements and general description. The male of this species is distinctive because of a long body, 0.847 mm. in length; onchiostyle measuring 78 microns long; a pair of lateral pores near the lip region; a thick-walled bursa; only two ventromedian supplementary papillae which are confined to bursal area; striated spicules measuring 68 microns in length; and an almost terminal anus. Its female can be distinguished by a long body and onchiostyle, anteriorly placed excretory pore and an almost terminal anus.

Trichodorus acaudatus comes closest to *T. atlanticus* Allen, 1957 but can be distinguished from it by the absence of a ventromedian oesophageal papilla, presence of lesser number of supplements, shorter and striated spicules and the presence of a pair of lateral pores behind the lip region.

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*The soil was kindly referred to us by Mr. P. S. Narayanaswamy of the Agricultural College and Research Institute, Coimbatore (South India).

Trematodes from Turkey with the Descriptions of *Creptotrema mülleri*, n. sp. and *Phagicola micracantha*, n. sp.*

WILLIAM H. COIL and ROBERT E. KUNTZ

The U. S. Naval Medical Research Unit No. 3, Cairo, was invited by the Minister of Health of the Turkish Government to send a field group to Turkey for a period of 2 months during the summer of 1953. This group, consisting of two investigators (a medical zoologist and a parasitologist) and three assistants, was designated as the U. S. Naval Medical Reconnaissance Group to Turkey. The studies resulting from this trip are a continuation of geo-medical and biological investigations in the Near East with emphasis on the parasites of man and animals, and their host-parasite relationship.

The parasites reported in these studies were collected at the following localities: (1) Lake Abant, a mountainous area with an altitude of 1200 to 4500 feet; (2) Sapanca, a small village on the shores of a freshwater lake in the fertile coastal plain a few miles inland from Istanbul; (3) outskirts of the port city of Istanbul.

This paper is the second report on the trematodes collected in Turkey by the junior author.

MATERIAL AND METHODS

The majority of the hosts were captured alive and were examined shortly after death. After the preparation of the host for identification purposes, the viscera were removed from each carcass. Each system was examined separately with the aid of a dissecting microscope during and after maceration with small needles and splinter forceps. Another examination was made after tissues had been shaken in a stoppered flask or pint jar in several changes of water. Frequently small worms were thus removed from the clean sediment.

All helminths were killed by quick immersion into hot water and then were transferred to dishes with FAA (formalin-acetic acid-alcohol) fixative. After 8 to 15 hours the specimens were transferred to procaine tubes and larger vials with 70 per cent alcohol plus 2 per cent glycerine. Harris' haematoxylin and damar were used in the preparation of the whole mounts. Figures 1 and 2 were made with the aid of a microprojector and figure 3 was made with the aid of a camera lucida. All measurements are in millimeters.

FAMILY ALLOCREADIIDAE Stossich, 1903

Creptotrema mülleri, n. sp. (Figure 1)

DIAGNOSIS: with characters of genus. Elongate distomes up to 4.1 long. Cuticular spines not observed. Width 0.82-0.92 at widest part. Oral sucker terminal with opening directed somewhat ventrally; width 0.32-0.34. Oral sucker with prominent ventro-lateral processes or "ears." Prepharynx 0.22-

*Studies from the Department of Zoology, University of Nebraska No. 309 (William H. Coil).

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The authors are indebted to Harry Hoogstraal, Head, Department of Medical Zoology, Naval Medical Research Unit No. 3, Cairo, for assistance in obtaining animals examined and for their identification. Dr. A. L. Rand, Curator of Birds, Chicago Natural History Museum, has provided the identification for birds and Dr. Robert F. Inger, Curator of Reptiles, of the same institution, has provided identifications for the reptiles and amphibians. The junior author is also indebted to Dr. Edip Beker, representative of the Turkish Ministry of Health who added greatly to the success of the trip acting as the energetic interpreter and as the liaison official.

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0.24 wide. Esophagus broad, thin-walled, extending to acetabulum. Exact extent and disposition of ceca not visible due to vitelline follicles. Acetabulum 0.510-0.58 wide, located about one-fourth of body length from anterior end. Testes in tandem with anterior one in middle of body; size 0.42-0.51 x 0.44-0.51. Cirrus sac large, extending from just back of pharynx to past

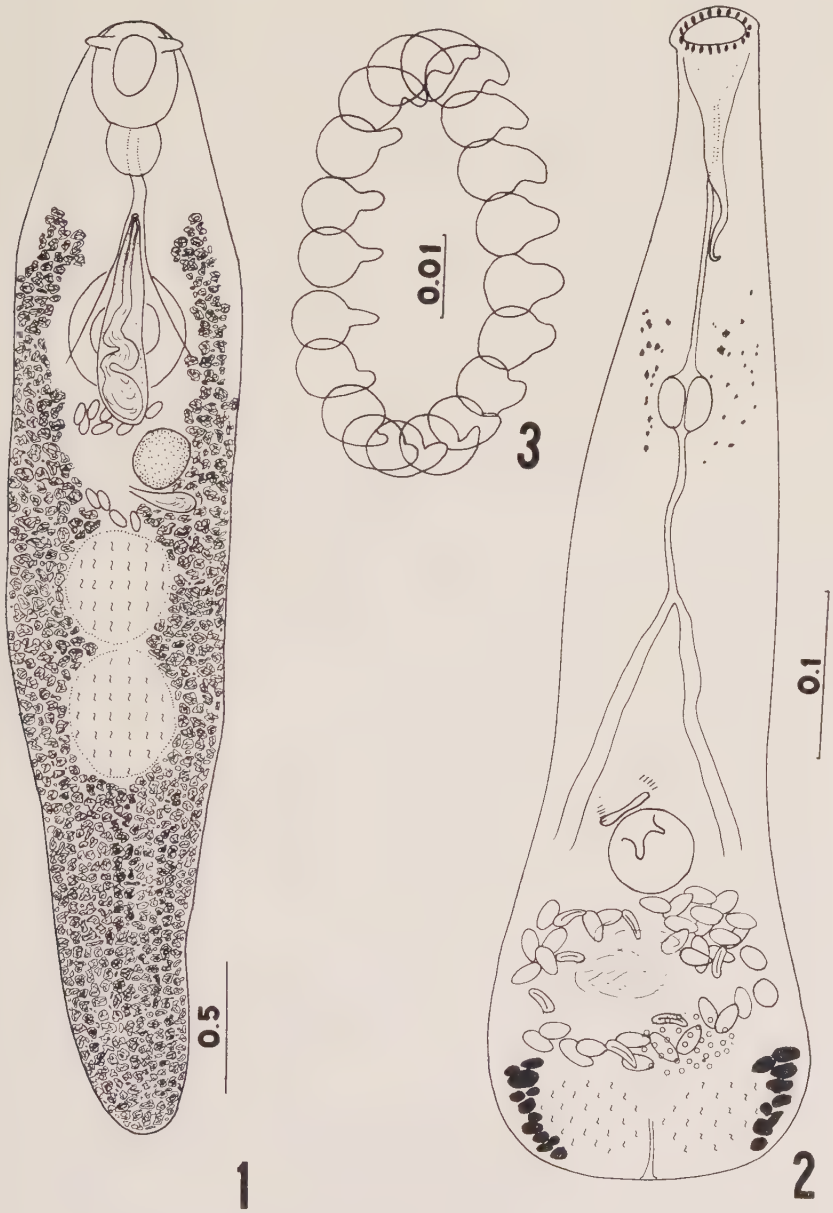

Figure 1. Ventral aspect of *Creptotrema mülleri.*
Figure 2. Dorsal aspect of *Phagicola micracantha.*
Figure 3. Cephalic spines of *Phagicola micracantha.*

posterior margin of acetabulum. Internal seminal vesicle large, convoluted, occupying posterior half of cirrus sac. Cirrus long, coiled, muscular, spines not visible. Genital pore slightly muscular, located slightly posterior to pharynx on midline. Vagina thin-walled, dorsal to acetabulum and close to cirrus sac. Ovary lateral, just posterior to end of cirrus sac; 0.18 x 0.24 in size. Seminal receptacle lateral, adjacent, and posterior to ovary; about 0.83 wide. Uterus anterior to testes with most eggs between acetabulum and anterior testis. Vitelline follicles very dense, completely filling body behind posterior testis, lateral follicles extending to level of genital pore. Eggs 0.041-0.043 x 0.072-0.076 in size.

Host: Speckled trout (scientific name unknown).

Site of infection: Kidney.

Locality: Lake Abant, Turkey.

Type specimen: Holotype in the Helminthological Collection of the U.S.N.M., No. 39143.

The genus *Creptotrema* appears to be a highly artificial group. The "ears" on the oral sucker are the unique feature common to the members of this genus. *C. mülleri* differs from *C. funduli* and *C. creptotrema* by possessing testes in tandem and by the presence of a long, posterior extension which is densely filled with vitelline follicles.

Family Lecithodendriidae Odhner, 1910

Prosotocus confusum (Looss, 1894)

Host: *Rana ridibunda.*

Site of infection: Small and large intestine.

Locality: Izmit, Turkey.

Specimens: U.S.N.M. Helminthological Collection, No. 39144.

Family Echinostomatidae Looss, 1902

Chaunocephalus ferox (Rud., 1795)

Host: *Ciconia ciconia*

Site of infection: Small intestine.

Locality: Izmit, Turkey.

Specimens: U.S.N.M. Helminthological Collection, No. 38298.

Family Plagiorchiidae Luhe, 1910

Telorchis solivagus Odhner, 1902

Host: *Clemmys caspica rivulata.*

Site of infection: Small intestine.

Locality: Istanbul, Turkey.

Specimens: Helminthological Collection of the U.S.N.M., No. 39145.

The specimens of this trematode studied here are somewhat larger than those reported, but in view of the large variations found in this species, it seems very likely that the specimen here is conspecific with the described species.

Family Heterophyidae Odhner, 1914

Phagicola longicollis Kuntz and Chandler, 1956

Hosts: Kite (*Milvus migrans*) and domestic cat.

Site of infection: Small intestine.

Locality: Istanbul, Turkey.

Specimens: Helminthological Collection of the U.S.N.M., No. 39146.

Metagonimus ciureanus (Witenberg, 1929)

HOST: Domestic cat.
SITE OF INFECTION: Small intestine.
LOCALITY: Sapanca, Turkey.
SPECIMENS: U.S.N.M. Helminthological Collection, No. 39147.

Phagicola micracantha, n. sp. (Figures 2 and 3)

DIAGNOSIS: with characters of genus. Very small, elongate, pyriform-shaped distomes. Cuticle covered with scale-like spines arranged in quinque-fashion; extending about to level of testes. Spines reduced in size progressively toward posterior. Body 0.73-0.83 long and 0.18-0.31 wide at greatest width (posterior quarter). Oral sucker 0.040-0.042 wide, located terminally in excellently preserved specimens (i.e., with cuticular spines intact). Anterior end armed with single row of 20 comma-shaped spines, 0.0104-0.0112 long. Oral sucker with elongate, slender appendage 0.084-0.095 long, curved or S-shaped at distal end. Prepharynx 0.16-0.20 long. Pharynx 0.034-0.045 wide. Moderate esophagus present. Ceca extend to posterior region, walls not especially thick. Genital pore adjacent, anterior, and slightly lateral to acetabulum. Genital sac in region of gonotyls appears muscular. Gonotyls with 5 or 6 "chitinous bars" or "gland cells," not apparent in all specimens. Acetabulum 0.44-0.54 wide. Testes oval or ellipsoidal, 0.053-0.061 in greatest dimension, symmetrically situated almost at posterior end of body. Seminal receptacle centrally located, posterior to acetabulum, apparently consisting of at least two large compartments. Vagina not apparent. Uterus with many eggs, several transverse slings between ovary and acetabulum. Ovary oval, located just anterior to testis, smaller than testes, not always visible in our specimens. Vitelline follicles of moderate size, located along lateral edges of testes. Eggs 0.0209-0.0226 x 0.0130-0.0136, with prominent operculum.

HOST: Turtle (*Clemmys caspica rivulata*), Kite (*Milvus migrans*).
SITE OF INFECTION: Small intestine.
LOCALITY: Istanbul, Turkey.
HOLOTYPE: U.S.N.M. Helminthological Collection, No. 39148.

Phagicola micracantha resembles both *P. longicollis* Kuntz and Chandler, 1956 and *P. inglei* Hutton and Sogandares-Bernal, 1958. These three species have in common the oral or cephalic spines which are oval with a narrow rounded tip. Comparisons of some of the important features of these species can be made from the following chart:

	micracantha	*longicollis*	*inglei*
Cephalic spines			
number	20	14-15	19
size	0.0104-0.0112	0.017-0.018	0.017-0.022
Body length	0.78-0.83	0.61-1.0	1.1
Width of oral sucker	0.041-0.042	0.040-0.050	0.068
Width of acetabulum	0.044-0.054	0.030-0.048	0.072
Width of pharynx	0.034-0.045	0.032-0.053	0.055

It can be seen that the dimensions of the soft parts are close or overlapping, but the spines, which are not so sensitive to the techniques of preparation, are clearly different in both size and numbers in the three species. Kuntz et al, 1956 and Hutton et al, 1958 considered the species they described to be close to *P. longa* (Ransom, 1920) Price, 1932, a species which possesses cephalic spines of a slightly different shape.

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Studies in cysticercoid histology. I. Observations on the fully developed cysticercoid of *Hymenolepis diminuta* (Cestoda:Cyclophyllidea)*

MARIETTA VOGE

In recent years the cestode *Hymenolepis diminuta* has become one of the laboratory animals frequently used in investigations of many aspects of tapeworm biology. It is therefore surprising that no studies are available on the microscopic structure of the cysticercoid. Moreover, observations on cysticercoid structure of other species are either fragmentary or deal primarily with gross morphology. The present report contains observations on the histology of fully developed, normal cysticercoids of *Hymenolepis diminuta,* and represents the first study of a series dealing with normal cysticercoid structure in different tapeworm species.

Grateful acknowledgement is made to Mrs. Nora Liu for technical assistance.

MATERIALS AND METHODS

Cysticercoids of *Hymenolepis diminuta* were grown in the confused flour beetle *Tribolium confusum* at 30°C, maintained on enriched flour. For studies on the fully developed cysticercoid, beetles were dissected in normal saline at least 10 days after infection. Five, six, or seven-day old cysticercoids were obtained for preliminary studies on the development of certain tissues. Live material was observed under the phase microscope for the presence of flame cells. For the preparation of sections, cysticercoids were fixed in different fixatives (Bouin's, Zenker's, acid alcohol and formalin, etc.) depending on the stain to be used. Cysticercoids were embedded in paraffin and sections cut at 10 microns. Stains used were Mallory's aniline blue collagen stain as directed by Gridley (1953), Mallory's phosphotungstic acid hematoxylin, methyl-green pyronin, Langeron's alizarin red S, Mayer's hemalum, Feulgen, Bodian copper protargol as described by Lillie (1954), and Davenport's protargol method (Davenport *et al,* 1939). Gomori's trichrome was used with Bouin's-fixed material as outlined by Lillie (1954), except that light green was substituted for fast green and the alum hematoxylin was omitted.

DESCRIPTION

The appearance and overall organization of the fully developed cysticercoid of *Hymenolepis diminuta* are illustrated in Figure 1 which represents a longitudinal section of this organism. The names employed to identify the different tissues or areas were selected for the purpose of description only and, with few exceptions, are not meant to suggest similarities in organization with adult tapeworms.

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The outermost layer or the external membrane (Fig. 1) is a thin and relatively delicate structure surrounding the whole cysticercoid. A surface view reveals thickened lines arranged in parallel fashion (perhaps an artifact) or an irregular network. In cross-section one observes that the membrane consists of two easily separable sheets, the outermost being very fragile and easily damaged or lifted above the second one beneath it. The space between these two layers reacts positively with fat stains (Voge, 1960). Beneath the external membrane, there are irregularly distributed acellular deposits which vary considerably in size and shape, and stain a uniform red with Mallory's aniline blue stain.

Next to the external membrane and in contact with its inner component is the peripheral layer which surrounds the body of the cysticercoid but does not extend into the tail. It consists of numerous hair-like processes arising from the surface of a single layer of cells (Figs. 1 and 2). These cells are flask or pear-shaped, or they may appear nearly rectangular depending on the section and perhaps the method of preparation. The cytoplasm of these cells, as well as the hair-like processes stain a deep blue with Mallory's aniline blue, while the small cell nuclei stain red. Among the hair-like processes there are scattered small granules which stain an intense red with Mallory's aniline blue but are not revealed with Mayer's hematoxylin or Gomori's trichrome. The latter two stains are not satisfactory for the demonstration of the peripheral layer.

Beneath the peripheral layer are situated relatively large cells which, in fully grown cysticercoids, stain weakly so that the cell borders are not always distinct. The nuclei are relatively large with well defined nuclear membranes and contain a small spherical mass of chromatin. These cells make up most of the intermediate layer (Fig. 1) and are present in the body of the cysticercoid but do not extend into the tail. The intermediate layer also contains a number of relatively small, deeply staining nuclei. Whether these represent remnants of degenerating cells or nuclei of other cell types could not be determined. Some of the nuclei are associated with elongated strands of cytoplasm and resemble the nuclei commonly observed in the tail parenchyma.

Beneath the intermediate layer is the fibrous layer. It is composed of a dense arrangement of fine fibers with interspersed elongated nuclei. Fibrous processes from this layer pass through the intermediate layer (Fig. 1) and connect with the cells of the peripheral layer (Fig. 3). Moreover, a dense network of fibers extends posteriorly towards but not into the tail. With Mallory's aniline blue and Mallory's phosphotungstic hematoxylin all fibers stain a brilliant blue or pink to red, while the nuclei appear red, or purple. Silver stains are useful in demonstrating the fibrous processes. All other stains used did not stain the fibers.

Beneath the fibrous layer is a narrow layer of very elongate cells which in Figure 1 is referred to as the lining of the cysticercoid cavity. The nuclei are definitely associated with strands of cytoplasm which do not stain as intensely as the fibers of the fibrous layer, but otherwise resemble them closely. Some of these cells are in contact with the narrow strip of dense tissue surrounding the scolex.

The scolex is bordered by the cuticle which also lines the tissue immediately surrounding it (Fig. 1). The scolex consists of densely packed cells as evidenced by the presence of a large number of nuclei. The suckers are fully developed and their musculature distinct. In living material one observes

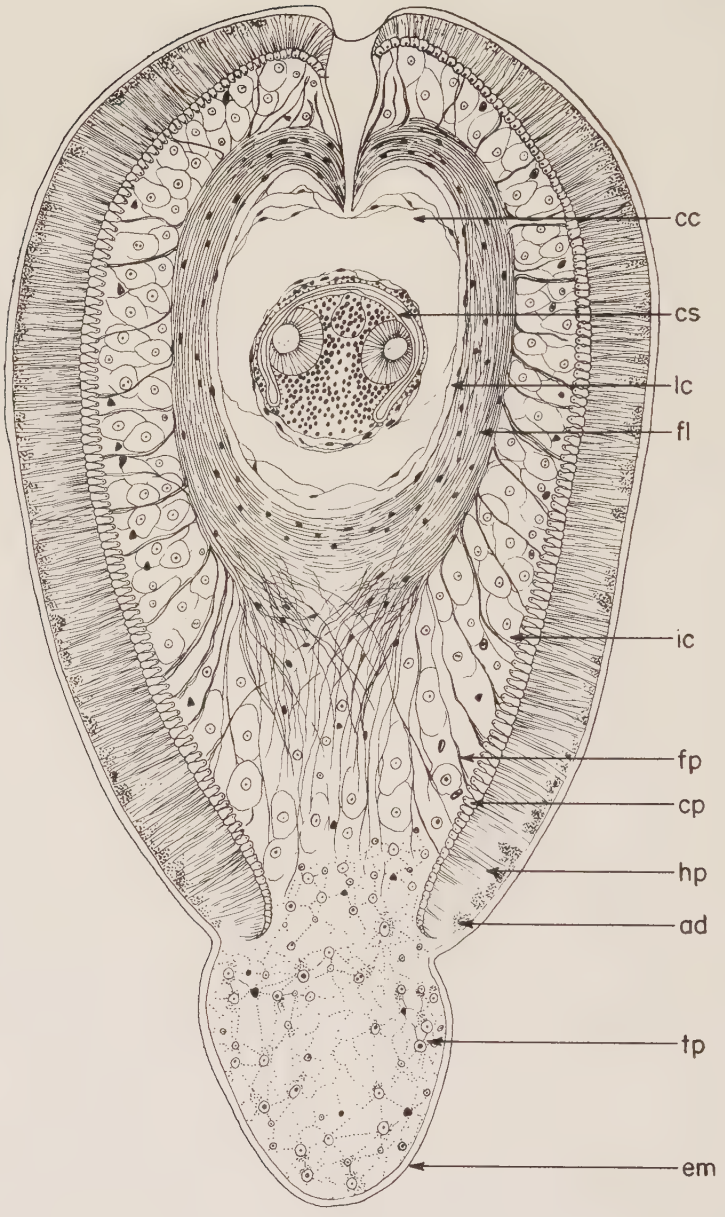

Figure 1. Free-hand drawing of longitudinal section of *Hymenolepis diminuta* cysticercoid showing relationships of different tissues in the fully developed organisms; ad: acellular deposits, cc: cysticercoid cavity, cp: cells of peripheral layer, cs: cuticle of scolex, em: external membrane, fl: fibrous layer, fp: fibrous processes of fibrous layer, hp: hair-like processes of peripheral layer, ic: intermediate cell layer, lc: lining of cysticercoid cavity, tp: tail parenchyma.

flame cells situated close to the outer margins of the suckers. Flame cells were not seen in any area other than the scolex. Excretory ducts could not be traced either in living material or in sections.

The cysticercoid tail consists of a fine irregular network of cells which are referred to as the tail parenchyma (Fig. 1). The nuclei differ in size and structure, one type being relatively large, the other smaller and staining more heavily. The same types of nuclei were observed in the intermediate layer of the body of the cysticercoid. The parenchyma is seen to extend beyond the body-tail junction into the posterior portion of the cysticercoid body.

Preliminary observations on developmental stages of *H. diminuta* cysticercoids have shown an organization considerably different from that of fully developed cysticercoids. The hair-like processes of the peripheral layer are not visible until the 8th day of development. They increase in extent and length thereafter. The cells from which they originate, however, are discernible before that time. Some of the fibrous connections between these cells and the fibrous layer are already established by seven days development but the fibrous layer proper is much less extensive than it is in older cysticercoids. Furthermore, the staining reaction with Mallory's aniline blue in the fibrous layer varies from blue to purple, indicates perhaps the presence of transitional cell or tissue stages. Individual fibers are not sharply delineated as they are in older cysticercoids. The cell borders of the intermediate layer are sharply defined and their cytoplasm stains intensely. Some of these cells have long and narrow processes which extend to the area just beneath the external membrane. These processes are not apparent in older cysticercoids.

DISCUSSION

The apparent complexity of organization of the fully developed cysticercoid of *Hymenolepis diminuta* is perhaps surprising when one considers the ultimate fate of all tissues other than those within the cavity. As far as known, only the scolex and the tissue immediately surrounding it will participate in the formation of the adult tapeworm. All other tissues are apparently discarded upon entry into the final host. Hence it may be assumed that the tissues outside the cysticercoid cavity function in some manner in the intermediate host and are perhaps essential for the maintenance and protection of the fully developed cysticercoid. With respect to passage into the final host it could be argued that protection from the host's digestive enzymes should not necessitate such extensive histologic differentiation.

While a discussion of the possible function of the different tissue layers would be premature, a consideration of the affinities and relationships of the cysticercoid tissues with those of the adult tapeworm will be presented. As already mentioned, the names assigned to the various layers surrounding the cysticercoid cavity (Figs. 1-3) were purposely general ones. From observation of the fully grown cysticercoid it is not certain that these tissues can be discussed in terms of what is known about the histology of adult tapeworms. The external cysticercoid membrane and the seemingly highly specialized peripheral layer superficially bear no resemblance to the cuticle of the scolex within the cysticercoid cavity. The large cells of the intermediate layer do not resemble any of the cells described in adult tapeworms. However, in developmental stages of the cysticercoid these cells resemble the so-called epithelial cells situated beneath the cuticle (see Wardle and McLeod, 1952, p. 14) or the subcuticlar cells (Hyman 1951, p. 319). The fibrous layer of

the cysticercoid has a staining reaction quite similar to that of collagen fibrils in mammalian tissue but does not resemble any of the tissues described in adult tapeworms. Only the tail parenchyma of the cysticercoid resembles the description given of parenchyma in adults, although the cysticercoid tail does not contain any of the large cells described for adult parenchyma.

Preliminary observations indicate the presence of distinct differences in the histology of different species of hymenolepidid cysticercoids. The hair-like processes in the peripheral layer of *H. diminuta,* for example, are absent in both *H. nana* and *H. citelli.* For a better understanding of cysticercoid structure, further study of fully grown cysticercoids of different cestode species, as well as cysticercoid developmental stages must be undertaken. Knowledge of the progressive differentiation of cysticercoid tissue is essential for interpretation of the histologic structure of fully grown forms and for comparison with tissues of adult tapeworms. Investigations of some of these problems, including additional studies on fully developed cysticercoids, are in progress.

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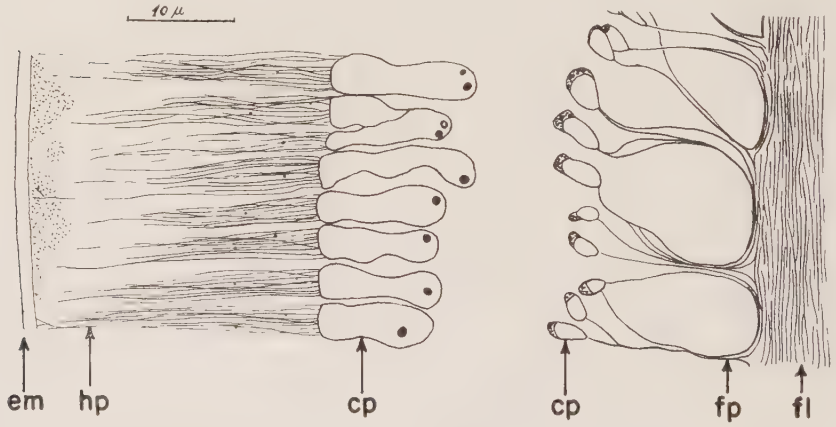

Figure 2. Camera lucida drawing of peripheral layer showing the cells (cp), the hair-like processes (hp), and the external membrane of the cysticercoid (Mallory's aniline blue stain).

Figure 3. Camera lucida drawing of fibrous layer (fl) and the fibrous processes (fp) which are connected with the cells of the peripheral layer (cp); appearance of the latter varies considerably depending on orientation of section and on method of preparation (protargol). Cells of intermediate layer are not shown.

A Method for the Concentration of Nematodes for Mounting from the Baermann Apparatus*

BERT M. ZUCKERMAN**

In the course of making routine nematode extractions from soil by Christie and Perry's (1951) modification of the Baermann technique, it became apparent that a rapid method for concentrating the nematodes drawn from the Baermann funnel for mounting would be of great utility. A technique whereby a representative sample of the nematodes drawn from the Baermann extract is rapidly concentrated into a single drop of water on a slide prior to microscopic examination is described in this paper. The object of the method is to sample without bias rather than to get all of the nematodes concentrated.

The sample is allowed to stand in the Baermann funnel for at least 24 hours to provide time for the less active nematodes to work themselves through the cloth. An aliquot of 10-15 ml is drawn from the Baermann funnel into a beaker and the nematodes relaxed by heating in an incubator at 60° C for five minutes. The contents of the beaker are then transferred to a 125 ml separatory funnel. A glass tube tapered to an opening of 1 mm is attached to the base of the funnel by means of rubber tubing. The liquid is passed slowly through an inverted 250 mesh sieve, the rate of flow being governed by the glass stopcock of the separatory funnel (Fig. 1,A). Following this operation the sieve is returned to its normal position and adjusted above a microscope slide until the spot where the water had passed through is centered over the slide. A drop of killing and fixing solution is passed through the mesh at the same spot and falls to the slide, carrying with it a large number of the nematodes collected on the screen. The addition of cover slip and sealing compound complete the making of the slide.

The refinements described below aid in the application of this method. A ring of ZUT slide sealing compound† ½ inch in diameter applied to the upper and lower surfaces of the mesh helps to prevent the water from spreading as the liquid passes from the separatory funnel (Fig. 1,B). A drop of liquid detergent applied to the mesh in the center of this ring further aids the passage of the water through the sieve. Excess detergent is vigorously washed from the screen prior to passage of the Baermann extract. Any excess water hanging from the inverted sieve after this process is removed by applying suction with a medicine dropper. This step is particularly important and is done prior to reinverting the sieve. The microscope slide which is to receive the nematodes is supported in a position a few millimeters below the mesh on which the nematodes are concentrated. If the drop of killing and fixing solution adheres to the screen, the mesh is lightly depressed until it comes into contact with the slide. Under this treatment the drop of fixative can be easily transferred to the slide.

If it is desired to process several extracts in succession, the sieve must be thoroughly washed and then dried between samples. A lint-free cloth or filter paper is used to remove excess liquid from the pores of the screen. To obviate this difficulty a number of ZUT rings can be applied to the mesh,

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†Manufactured by Bennett Manufacturing Co., Salt Lake City, Utah.

then successive samples can be processed provided that the areas within the rings do not become wet or contaminated with nematodes from previous extracts.

In other tests a piece of bolting cloth, of about 300 mesh to an inch, was used to concentrate the nematodes *in lieu* of a sieve. The cloth was mounted in a wooden frame and ZUT rings applied to the upper and lower surfaces of the cloth as previously described for the sieve (Fig. 1,C).

An average of 61.2 percent of the nematodes from the Baermann extract were screened out when this method was applied in trials which involved 60 samples. These tests indicated that the sieve and the bolting cloth remove the nematodes from the Baermann extract with comparable efficiency. Some specimens are inevitably lost during the process of transferring the nematodes to the microscope slide; however, extracts which had a total population of 400 nematodes yielded slides which contained at least 50 specimens.

SUMMARY

A technique is described which provides for rapid concentration and mounting of nematodes from Baermann extracts. The nematode population is sampled without bias by this method. Theoretically all forms are represented on the completed slide in numbers proportionate to their abundance in the Baermann extract. The method is suggested as an adjunct to other soil and

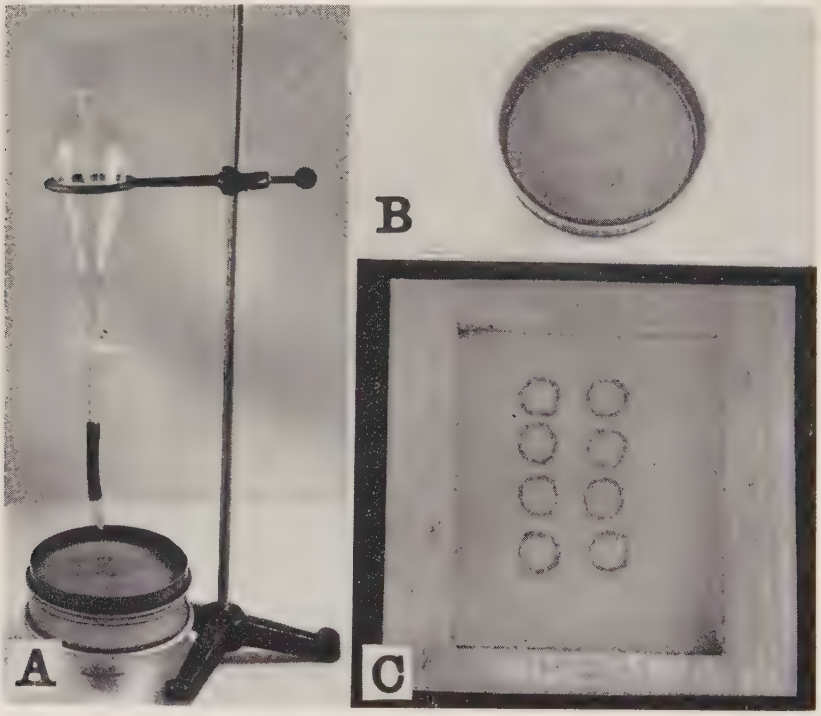

Figure 1,A) Baermann extract in separatory funnel prior to passage through an inverted 250 mesh sieve. The sieve is supported by a beaker which receives the processed extract; B) Sieve returned to normal position and microscope slide supported beneath the sieve to receive the concentrated nematodes; C) Bolting cloth mounted in wooden frame showing rings of ZUT applied to surface of the cloth.

plant tissue extraction techniques in which nematodes are separated from large quantities of liquid for microscopic examination.

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Studies on Two Gorgoderid Cercariae from Oklahoma with the Description of a New Species*

WILLIAM H. COIL

During the summer of 1958, sphaeriid mollusks were collected from the Red River drainage in southeastern Oklahoma in order to study the trematodes which parasitize them. Both cercariae which are discussed here were collected from the Blue River in Johnston County. This stream is springfed and quite cool, supporting several species of fish and amphibians which could serve as definitive hosts for gorgoderid trematodes.

The sphaeriids were removed from a sandy-mud bottom and placed in an ice cabinet for transportation to the laboratory. Living cercariae were studied with the aid of vital stains, and sections were stained with Harris' haematoxylin and A. G. E. Pearse's stain (Hotchkiss PAS, Celestin Blue, Toluidine Blue, and Orange G). This latter stain is especially good for unicellular glands.

I am indebted to Dr. Carl D. Riggs and his staff at the University of Oklahoma Biological Station for their unstinting efforts to help with my summer research problems. This study was supported, in part, by a National Science Foundation Grant-in-Aid.

Cercaria papillostoma n. sp. (Figures 1-3, 5)

DIAGNOSIS: Body measurements in millimeters of specimens fixed in neutralized formalin and mounted in piccolyte are: length of stylet 0.008, width of acetabulum 0.019-0.026, width of oral sucker 0.017-0.038, length of excretory bladder 0.034-0.039, distance between acetabulum and oral sucker 0.027-0.031, length of body 0.11-0.15. Suckers well-developed, active, covered with long thin "hairs" or setae. Some setate papillae on oral sucker. Characteristic "double" papillae on acetabulum arranged in hexagonal pattern. Three to four pairs of penetration glands. Ducts of penetration glands slightly lateral to median, passing around edge of oral sucker, then through it, opening laterad to stylet, cytoplasm and secretion granular. Excretory bladder surrounded by large, coarsely granular, cystogenous glands. Tail elongate, very large, swollen at anterior end into cercarial chamber which completely encloses body of cercaria. Distal part of tail swollen and wrinkled, tapering to a terminal knob which is adhesive. In live, active specimens, body enclosed in cercarial chamber, but in moribund specimens, body is half out or completely detached from tail. Tail extremely active, but not natatory. Body swollen laterally, posterior to acetabulum giving a superficial resemblance to the rhopalocercariae. Excretory system probably of the mesostomate type. Genital primordia present as undifferentiated clumps of cells.

HOST: *Pisidium compressum* Prime.

SITE OF INFECTION: Gills.

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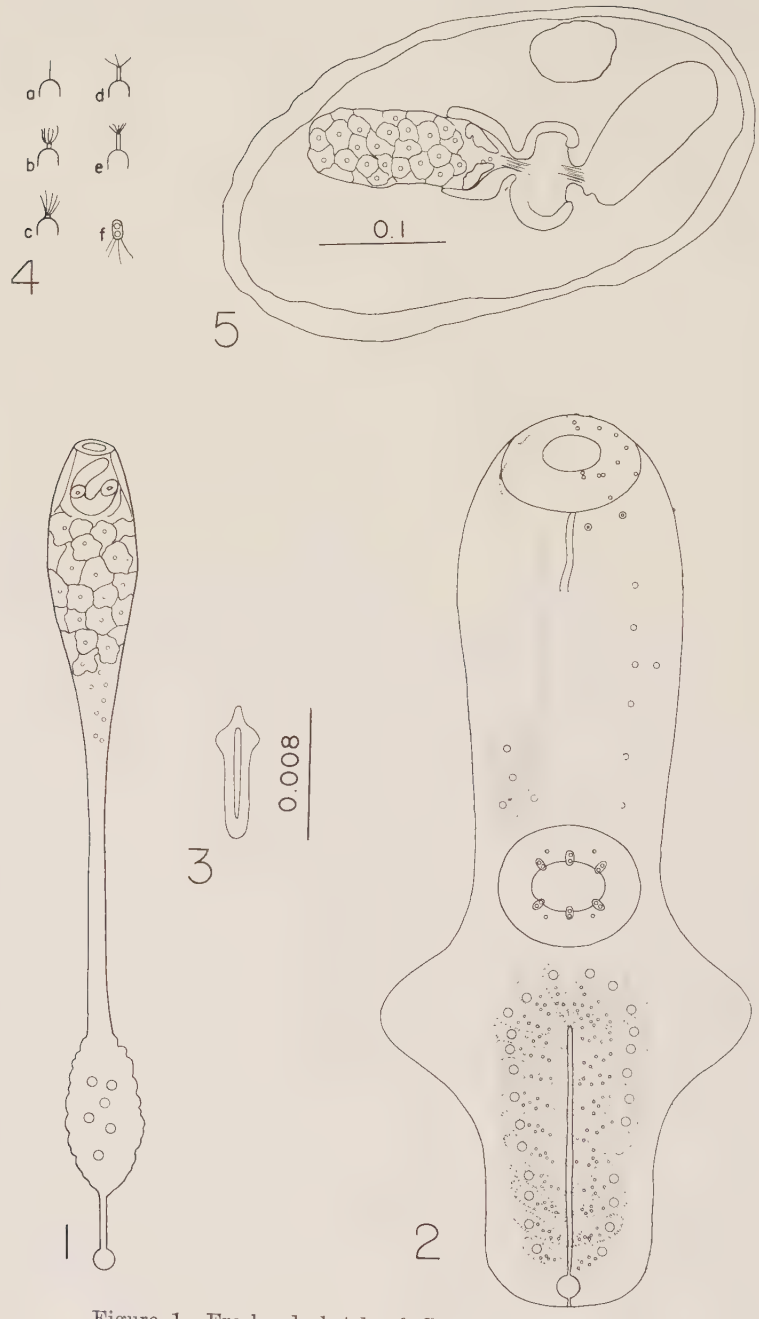

Figure 1. Freehand sketch of *Cercaria papillostoma.*
Figure 2. Freehand sketch of living specimen of *Cercaria papillostoma* under slight coverglass pressure.
Figure 3. The stylet of *C. papillostoma.*
Figure 4. Variations found in the setate papillae of gorgoderids.
Figure 5. Sporocyst of *C. papillostoma.*

LOCALITY: Blue River, Johnston County, Oklahoma, U.S.A.

Only two other gorgoderid cercariae have been reported as having four pairs of penetration glands (the cercaria of *Gorgodera cygnoides* (European) and *Cercaria coelocerca* Steelman, 1939). *Cercaria papillostoma* does not swim and it possesses an inordinately short stylet. Both of these characteristics will serve to differentiate it from the two species above.

One of the characteristic features of gorgoderid cercariae is the sensory papillae on the suckers and on the dorsal, ventral, and lateral body surfaces. The arrangement of these papillae has proved to be useful to systematics to separate similar species. Fischthal, 1950, used this method to differentiate among certain of the rhopalocercariae. Following his lead, I studied the arrangement of the papillae of Lake Erie gorgoderids (Coil, 1953, 1954a, and 1954b).

Although some, but not universal, attention has been given to the arrangement of the papillae, very little has been said concerning the structure of the individual papillae. Fischthal, 1950, described the "single" papilla with a refractile body and he also described the "double" papillae which are characteristically arranged in a hexagonal pattern on the acetabulum. He did not report or figure the setate papillae on the suckers (nor did I find them there) of the rhopalocercariae. Setate papillae were seen, however, on the posterior body surfaces of two Lake Erie rhopalocercariae.

While studying a second gorgoderid cercaria (close to the cercaria of *Gorgoderina attenuata*), it was noted that there were several types of setate papillae present. In an earlier study on a macrocercaria (*C. wabashensis* Coil, 1955), a papilla was noted on which the three setae were borne on a short stalk (Fig. 4, d). In that publication it was reported: "There is a striking similarity between this structure and the description by Sinitsin, 1905, of papillar nerves—'A nerve fiber enters the alveolus and when it reaches two-thirds of its distance it branches and ends in the wall of the alveolus' (from translation). It would appear, therefore, that the wall of the papilla disintegrates and leaves the nervous system visible." In view of this hypothesis, it is of considerable interest to find setate papillae of several types on a single cercaria. On the oral sucker of this second cercaria from the Blue River there was, on the anterior part of the oral sucker, a row of 4 papillae, each of which was almost identical to the one described for *C. wabashensis* (Fig. 4, d). In addition to this, on the posterior part of the oral sucker, there was a row of 4 papillae; the lateral ones were double (Fig. 4, f) and the median ones were single, and each of the four carried four long setae. Figure 4,a,b,c, and e depict some of the variations which were observed on the oral sucker.

From this additional information it is now apparent that we can no longer hold as tenable the hypothesis, concerning the nervous system, presented in connection with *C. wabashensis*.

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A New Species of Nematoda, *Cylindrocorpus erectus*, Associated with *Scolytus multistriatus* Marsh. in American Elm

Calvin L. Massey*

A new species of Cylindrocorpus was recovered in research work on nematodes associated with and parasitic on bark beetles in the central and southern Rocky Mountain regions. The species was taken from the egg and larval galleries of *Scolytus multistriatus* Marsh. in American elm.

Genus *Cylindrocorpus* Goodey, 1939

The genus *Cylindrocorpus* was proposed by Goodey in 1939 to include those species formerly described within the genus *Cylindrogaster* Goodey, 1927, *Cylindrogaster* having been preempted by Stal in 1855 for a Dermapterous insect. At the same time Cylindrocorpidae was proposed as a new family in place of Cylindrogasteridae Chitwood 1933 and includes the genera *Cylindrocorpus, Goodeyus, Myctolaimus* and *Longibucca.*

The genus *Cylindrocorpus* is defined by the following characteristics. Head with 6 forward pointing conical lips. Stoma elongate, composed of a cheilostom, protostom and telestom. Esophagus consisting of a cylindrical bulb, isthmus and terminal bulb. Ovaries paired, opposed and reflexed. Testes single, reflexed. Spicules paired, gubernaculum present. Cuticle of tail a narrow bursa supported by 9-10 pairs of papillae. Genotype *Cylindrocorpus longistoma* (Stef., 1922) Goodey, 1939.

Cylindrocorpus erectus, n. sp.

Female: .95 mm. a = 16, b = 5.5, c = 8.8, V = 47%.

Male: .77 mm. a = 17, b = 7.7, c = 19.8.

Cuticle thin, with fine longitudinal and transverse striations, the striations more apparent at midbody. Body of the female widest at the middle, sharply narrowing anteriorly from the region of the esophagus and posteriorly to a long finely pointed tail. Fig. 1 E. Body shape of the male more uniform in width throughout its entire length. Head with 6 forward pointing conical lips as in Fig. 1 A. Stoma elongate, one third the length of esophageal bulb, isthmus and terminal bulb combined, composed of a short cheilostom, a long protostom and a short telestom. Esophagus typical of the genus. Nerve ring slightly anterior to terminal bulb. Excretory pore not discernible in specimens examined. Female: Vulva a transverse slit only slightly protuberant, located approximately at the middle of the body length. Ovaries paired, opposed and reflexed, each uterus usually containing one egg at a time. Fig. 1 D.

Male: Testes single, reflexed, spicules as illustrated, Fig. 1 B. Cuticle of the tail expanded to form a narrow bursa supported by 9 pairs of papillae as in Fig. 1 C. Tail moderately short, finely spicate. Fig. 1 B, C.

Diagnosis: *Cylindrocorpus,* closely related to *curzii* Goodey 1935. Differs from that species in its stouter body form, *C. erectus,* both sexes, a = 16-17, *C. curzii,* female = 19-30, male = 23-28; shorter tail in the female, stouter tail of the male. Inflated cuticle of the tail in the male is supported by only 9 pairs of papillae.

Type localities: Albuquerque, New Mexico, and Fort Collins, Colorado.

Type specimens: Albuquerque Forest Insect Laboratory Collection. Slide numbers, 8 V, 25 G.

*Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Colorado State University, Fort Collins, Colorado.

The research reported was completed at the Station's Forest Insect Laboratory at Albuquerque, New Mexico.

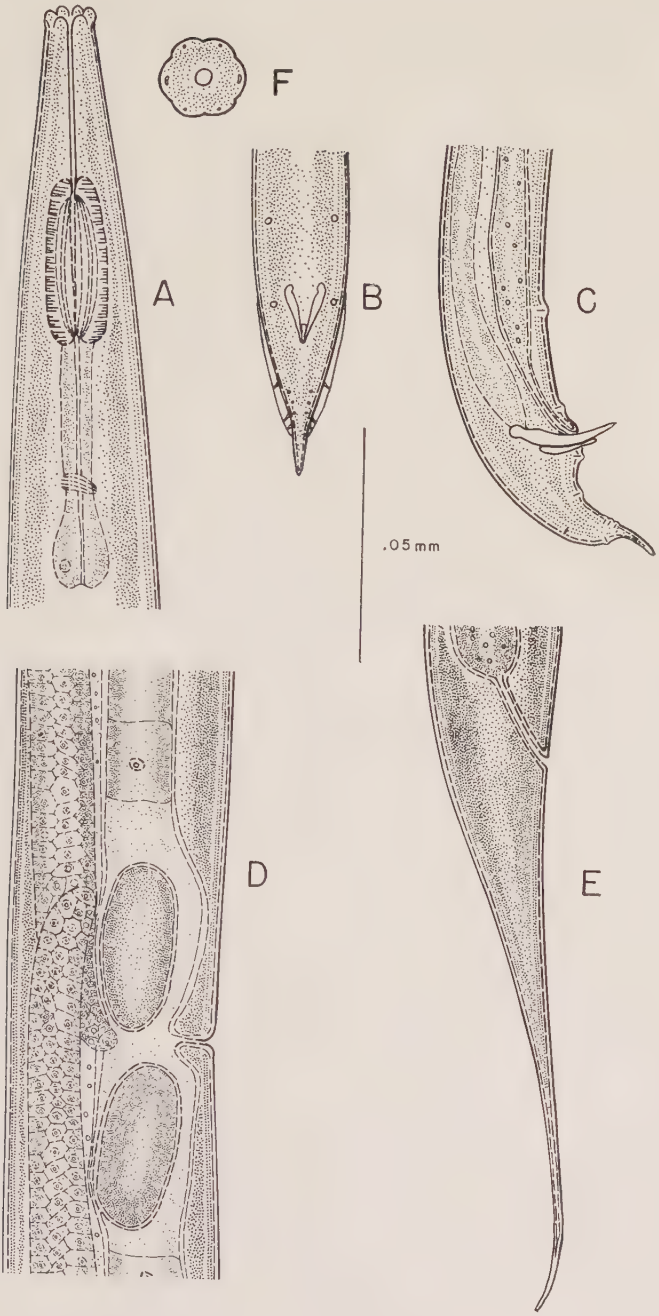

Figure 1. *Cylindrocorpus erectus* n. sp. A. Head; B. Male tail ventral view; C. Male tail lateral view; D. Midsection female; E. Female tail. F. Face view.

Other known species in the genus are *C. macrolaima* Schneider, 1866, *C. curzii* Goodey, 1935 and *C. longistoma* (Stef., 1922). Habitats of these species are widely varied. *C. longistoma* is known from rat faeces and ginger root, *C. macrolaimus* from moist soil and litter, and *C. curzii* from rotting cortical tissue of bitter cassava.

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Cercariae Belonging to the Opisthorchioidea

T. T. Dunagan*

The cercariae belonging to the trematode families of the Opisthorchioidea (Heterophyidae, Opisthorchiidae, Acanthostomatidae, Cryptogonimidae, Pachytrematidae, and Ratziidae) now include forms that do not have fin folds on the tail, yet retain the body characters previously associated with this group. Of necessity the earlier view, as expressed by Rothschild (1938, p. 170) that "So far only two types of cercariae have been definitely proved to belong to the superfamily Opisthorchioidea, namely those pertaining to the Pleurolophocerca and Parapleurolophocerca groups" must be changed to include cercariae not of this type, but whose adults occur in these families, a change that she undoubtedly expected. Cable (1943, p. 7) was perhaps the first to suggest such an enlargement. Later he (1956, pp. 411-512) put a zygocercous as well as four magnacercous cercariae in the Opisthorchioidea. However, to place all zygocercous and/or magnacercous cercariae in this group would be equally erroneous since many of them obviously belong in other superfamilies.†

At present to try to group the cercariae of this superfamily in a natural classification is almost impossible because of the poor cercarial descriptions and the shifting location of genera among families. Likewise, the problem of deciding which cercaria is synonymous with another is, as always, a point of much confusion. With the pleurolophocercous group this is especially true, since the cercariae complete their development in the snail after leaving the redia. The very common practice of cracking snails to obtain and study different stages of the parasite has undoubtedly led to the description of cercariae that would look much different if they had been allowed to emerge naturally.

The shortest, yet most complete, method of presenting a description of all the cercariae of this group is through the use of a key. I would be the first to admit that this method of presentation does not guarantee absolute identification, but it does quickly narrow a large field to a few selections. Obviously a key that attempts to include all described species of any particular group of cercariae can be no more accurate than the written descriptions available. That some of these descriptions are not completely correct was pointed out by Martin's recent work (1958). Even so, I prefer to use the author's description rather than speculate on his possible errors.

*Department of Biological Sciences, Purdue University, Lafayette, Indiana.
†Zygocercous and magnacercous forms such as the following marine cercariae: *C. equitator* Sinitsin 1911, *C. purpuracauda* & *C.* "F" Miller 1925, *C.* "T", "U", & "W" Miller 1929, and *C. caribbea* XVI, XVII, XVIII, & XIX Cable 1956 are not considered at this time.

The cercariae belonging to the superfamily Opisthorchioidea can be characterized as follows: Stylet absent; Oral sucker protrusible with acicular spines dorsal and adjacent to mouth opening; Ventral sucker absent or rudimentary; Body spined in part or completely; Biocellate; Excretory bladder thick walled; Primary excretory pores on margins of tail near body-tail junction; Digestive system usually undeveloped posterior to the pharynx; Development in simple redia in prosobranch gastropods; Encysting, as a rule, in fishes; Adults in piscivorous vertebrates.

Abbreviations used: BL, Body Length; BW, Body width; C & *C.*, Cercaria; CG, Cephalic glands (=Penetration glands); CGD, Cephalic gland duct openings; EXB, Excretory bladder; FC, Flame cells; FCF, Flame cell formula; FW, Freshwater; OS, Oral sucker; P, Pharynx; TL, Tail Length; VS, Ventral sucker. In numbering the acicular spines the first number given represents spines closest to the oral opening.

ARTIFICIAL KEY TO THE OPISTHORCHIOIDEA CERCARIAE

1. C without lateral fin folds on tail 18
   C with dorso-ventral and lateral tail fin fold 10
   C with lateral fin fold only; all have P except *C. tridonta* 2
2. CG in linear arrangement 3
   CG in cluster or unknown in a few cases 5
3. CG openings not in groups of 3 or 4 but in a single linear arrangement of 7 per side 4
   CG openings not in a single linear arrangement of 7 per side ..... *C. melanoides*
4. It is difficult to separate C in this group. C of *Haplorchis pumilo* differs in P size and location but at best this is a small difference.
5. Redia with "feet"; C with caeca reaching posterior part of body ..... *C. latucicauda*
   Redia without "feet"; Gut of C unknown below P 6
6. CG less than 10 7
   CG more than 10 8
7. CG = 2(6); EXB filled with numerous refractive granules ..... *C. translucens*
   CG = 2(8); EXB containing no refractive granules *C. indicae* XXXI
8. C developing in irregularly branched sporocyst without specific characters ..... *C. photifera*
   C developing in redia without "feet" 9
9. EXB with refractive granules; no P indicated; CG = 2(16-20) ..... *C. tridonta*
   EXB without refractive granules; P present; CG = 2(10 + 5) ..... *C. indicae* L
10. Ventral sucker absent 11
    Ventral sucker rudimentary; CG = 2(12 ?); P present(?); body spines not mentioned; C floats with the tail below the body ..... *C. quadripterygia*
    VS rudimentary; CG = 2(7) in linear arrangement to posterior body margin; P small; body completely spined ..... C of *Haplorchis pleurolophocerca*
    VS present; CG = 2(6); P present; body spines only to level of eyespots ..... *C. atomica*

11. CG = 2(10); CGD 6:4::4:6 ..... *C. parapleurolophocercoides*
CG = 2(7) or unknown in a few; intestinal caeca absent 12
12. Body completely covered with spines 14
Body covered with spines only on anterior end 13
13. CG = 2(8) or greater 13-A
CG = 2(7); P small 13-B
13-A. CG = 2(8); P small adjacent to OS; single row of 6 or less acicular spines ..... *C. parvomelaniae*
CG = 2(9); P present; lateral tail fin fold occupies proximal ⅓ of tail; acicular spines in one row of 20 ..... *C. indicae* VII
13-B. Acicular spines 4:5-8:5-8 ..... C of *Haplorchis taichui*
Acicular spines 6:5-8:5-8 ..... C of *Haplorchis yokogawai*
Acicular spines in single row of 20 (?); redia without "feet"; Body and tail form letter "S" when animal is floating *C. indicae* VIII
14. CG extending to or beyond anterior margin of tail stem 15
CG not extending posterior to anterior margin of tail stem 16
15. Lateral tail fin fold extending more than ½ TL; acicular spines in single row of 6 ..... *C. caribbea* X
Lateral tail fin folds extending 2/5-1/2 tail length; acicular spines in single row of 4-5 ..... C of *Euhaplorchis californiensis*
Acicular spines in 3 rows of 4:5:6 ..... *C. plotiopsis*
16. CGD 3:3::3:3; FC in groups of 3, 17 recorded/side ..... *C. pleurolophocercous* I
CGD 3:4::4:3; FC in groups of 3; FCF 2 [(3+3)+(3+3)] 17
17. Acicular spines in 3 rows 5-6:7-8:8-9 ..... C of *Stictodora tridactyla*
Acicular spines in 3 rows 5:5-6:6-8 ..... C of *Parastictodora hancocki*
18. Fin fold on tail dorso-ventral only 19
Fin fold absent or otherwise modified but never dorso-ventral 48
19. CG = 2(8) or more 20
CG = 2(7) or less 26
20. CG = 2(10); CGD 4:6::6:4 21
CG = 2(12); CGD 6:6::6:6 24
CG = 2(9); CGD 4:5::5:4 25
CG = ? 2(20); No P; intestinal caeca present ..... *C. caudata*
21. Ventral part of fin fold at least ¾ tail length ..... *C. caribbea* XIII
Ventral part of fin fold ½ or less as long as tail 22
22. FCF = 2 [(2+2)+(2+2)] 23
FCF = 2[(5) + (5 + 5 + 5 + 5)] ..... C of *Opisthorchis (tenuicollis) felineus*
23. P present ..... *C. orospinosa*
P absent ..... *C. bermudensis*
24. Body aspinose; caudal pockets indicated ..... *C. chromophila*
Body spinose to eyespot level beyond which "they are buried in the cuticle" ..... C of *Telogaster opisthorchis*
25. Body length 380-510 microns; redia in *Amnicola longiqua* C of *Cryptocotyle concavum*
Body length 120-200 microns; redia in *Littorina littorea* ..... C of *Cryptocotyle lingua*
26. CG = 2(7); CGD 3:4::4:3; dorso-ventral fin fold continuous for full length of tail or at least ¾ tail length 27
Dorsal fin fold longer than ventral fin fold 31

27. Tail not coiled when C at rest (living) but C floats in "S" shape 28
Tail coiled when C at rest (living) 30
28. Acicular spines in 3 rows; ventral tail fin fold narrower throughout most of its length than the dorsal fin fold 29
Acicular spines in 3 rows; 4-10-12; ventral fin fold as wide as dorsal fin fold only very briefly ..... C of *Metagonimus yokogawai*
Acicular spines not in 3 rows; ventral tail fin fold as wide as dorsal fin fold throughout tail length ..... C of *Exorchis oviformis*
29. CG in single linear arrangement per side ..... C of *Heterophyes aequalis*
CG not in linear arrangement; acicular spines 7:14:14; FCF = 2 [(3 + 3 + 3) + (3 + 3 + 3)] ..... *C. vogeli*
30. Anterior end not capable of secondary retraction ..... C of *Siphodera vinaledwardsii*
Anterior end capable of secondary retraction ..... C of *Metadena adglobosa*
31. All forms of pigment absent 52
Pigment found in eyespots 32
32. CGD 3:4::4:3 33
CGD not 3:4::4:3 34
33. Dorsal tail fin fold not attached near tail body junction 35
Dorsal tail fin fold attached near tail body junction; fin fold extends for at least 5/6 of tail length 34
34. CG not in a linear arrangement ..... *C. opacocorpa*
CG in linear arrangement; FCF = 2 [(3 + 3 + 3) + (3 + 3 + 3)]; CG anterior to EXB ..... C of *Heterophyes* sp. M & K 1955
CG in linear arrangement extending to posterior body margin; FCF = 2 [(3 + 3 + 3) + (3 + 3 + 3)] ..... C of *Stellantchasmus falcatus*
35. Dorsal tail fin fold extends ⅓ tail length ..... *C. semicarinatae*
Dorsal tail fin fold extends beyond mid-length of tail 36
36. Body completely covered with small spines which may be larger toward the anterior extremity 37
Body covered with spines only on the anterior end 40
Body spines absent; a few "bristles" may be present ..... C of *Centrocestus formosanus*
37. Lobe-like proximal ventral fin fold of greater width than diameter of tail ..... C of *Caecincola parvulus*
Ventral fin fold without proximal lobe 38
38. Intestinal caeca present extending to anterior EXB ..... *C. pleurolophocercous* II
Intestinal caeca absent; P present 39
39. Described from FW snails; TL 416-450 microns ..... C of *Apophallus brevis*
Described from brackish and salt water snails; TL 285-315 microns ..... *C. caribbea* XI
40. EXB shows a distinct transverse constriction; VS present but small ..... *C. constrictovesica*
EXB without transverse constriction 41
41. P present (well developed or rudimentary) 42
P absent 43

42. Body has only acicular spines; P rudimentary ..... *C. indicae* III
Anterior half of body spinose; P developed; FCF = 2 [(2 + 2 + 2) + (2 + 2 + 2)] ..... C of *Phocitremoides ovale*
Body spined to level of pharynx; FCF = 2 [(3 + 3) + (3 + 3 + 3)] ..... C of *Opisthorchis sinensis*
43. EXB round ..... C of *Metagonimus yokogawai*
EXB decidedly U or V shaped ..... *C. floridensis*
44. Intestinal caeca present; body void of protoplasmic hairs 45
Intestinal caeca absent; P present but may be rudimentary 46
45. Gut without swollen caeca; CGD 4:4::4:4; CG located from eyespots to genital anlagen ..... C of *Apophallus venustus*
46. CGD 5:2::2:5; acicular spines in 3 rows 4:5:4; long delicate setae more prominent on anterior end ..... *C. caribbea* XII
CGD not 5:2::2:5 47
47. CGD 3:4::4:3; body spined only to level of eyespots ..... C of *Metorchis intermedius*
CGD 3:5::5:3; fine spines completely cover body ..... C of *Metorchis conjunctus*
48. Tail with ventral fin fold only ..... C of *Metagonimoides oregonensis*
Tail without a fin fold; not magnacercous or zygocercous 49
Tail with the fin fold arranged in the form of a helix 51
Tail without a fin fold; tail of magnacercous or zygocercous type 53
49. CG = 2(7); CGD = 3:4::4:3 50
CG = 2(4); CGD = 2:2::2:2 ..... C of *Centrocestus armatus*
CG = 2(8); VS well developed; tail with short "setae-like" extensions from "cuticular" elevations ..... *C. bithyniella*
50. EXB round; acicular spines 4:11:13 ..... C of *Metagonimus takahashi*
EXB "V" shaped; acicular spines 3:4:9 ..... C of *Pseudexorchis major*
51. Fin fold does not extend around tip of tail ..... *C. britsiae*
Fin fold does extend around tip of tail ..... *C. sigmoida*
52. CG = 2(6); terminal tail attachment ..... C of *Euryhelmis monorchis*
CG = 2(7); ventral tail attachment ..... *C. nicobarica* I
53. Zygocercous; Body completely spined; acicular spines 12:12 ..... *C. caribbea* XVI
Magnacercous type tail 54
54. Enlarged cuticular spines ventrolateral to mouth; CG 2(7) 55
Spines ventrolateral to mouth not enlarged 56
55. Tail abruptly enlarged with black pigment near base; anterior half of body spined ..... *C. caribbea* XVII
Tail not abruptly enlarged; purple pigment present in tail; body completely spined ..... *C. caribbea* XVIII
Body always wider than tail; tail not noticeably pigmented; anterior half of body spined ..... *C. caribbea* XIX
56. P present; purple pigmentation in tail ..... *C. purpuracauda*
P absent; tail transparent ..... *C. equitator*

The following cercariae have not been included in the above key. In most cases, the characters I have had to use have not been adequately described for these. It is surprising that some of them are so well known and yet so poorly described morphologically.

C. picta Faust 1924 (Yoshida 1917, Cercaria 'b'; Kobayashi 1918, Cercaria 'E'; Ando 1918, Cercaria sp. XI; Kobayashi 1922, Cercaria 'B') BL 86-90 microns, BW 40 microns.

CERCARIA	AUTHOR DESCRIBING C	SNAIL HOST—LOCALITY
C. fulvopunctata	Ercolani 1882	*Bythinia tentaculata*—Italy
C. elegans	Bregenzer 1916	*Bythinella dunkeri*—Germany
*C. quadripterygia**	Sinitsin 1911	*Hydrobia ventrosa*—Black Sea
*C. equitator**	Sinitsin 1911	*Cerithiolum exille*, Black Sea
C of *Pygidiopsis summus*	Nishio 1915	?—China, Japan
C. indicae VIII	Sewell 1922	*Melanoides tuberculatus*—India
		Acrostoma variable
		Digoniostoma ceramcopoma
C. indicae VII	Sewell 1922	same as *C. indicae* VIII
C. indicae L*	Sewell 1922	*Amnicola travancorica*—India
C. indicae III	Sewell 1922	*Melanoides tuberculatus*—India
		M. scabra, M. lineatus
C. indicae XXXI*	Sewell 1922	*Digoniostoma ceramcopoma*—India
C. photifera	Faust 1922	*Viviparus polyzonatus*—China
C. chromophila	Faust 1922 (Osafaune 1898)	*Melania ebenia*—China, Japan
C. latucicauda	Faust 1924	*Bythinia striatula*- China
C. cordata	Faust 1924	*Melanoides tuberculatus*—China
C of *Centrocestus formosanus*	Nishigori 1924 (Martin 1958)	*Melania tuberculatus*—China, Japan
C. picta	Faust 1924 (Yoshida 1917)	*Bulinus* sp.—Korea
C. benigna	Faust 1924 (Nakagawa 1915)	*Melania libertina*—Formosa
*C. purpuracauda**	Miller 1925	*Bittium eschrichtii*, Washington
C. "F"*	Miller 1925	*Cerithium litteratum*, Washington
C of *Haplorchis pumilio*	Faust & Nishigori 1926	*Melania reiniana*— Formosa
C of *Haplorchis taichui*	Faust & Nishigori 1926	*Melania obliquegranulosa*- Formosa
C. translucens	Faust & Nishigori 1926	*Bithynia striatula*- China
C. tridonta	Faust & Nishigori 1926	*Bithynia sinensis*—China
C of *Opisthorchis sinensis*	Faust & Khaw 1927 (after Yamaguti 1935)	*Melania* sp., *Bythinia* sp.— China
C. parvomelaniae	Tubangui 1928	*Melania* sp.—Philippine Isl.
C. floridensis	McCoy 1929	*Cerithium litteratum*—Florida
C. "T", "U", & "W"*	Miller 1929	*Cerithium litteratum*, Washington
C of *Pseudexorchis major*	Takahashi 1929	*Melania* sp.- Japan
C of *Metagonimus yokogawai*	Takahashi 1929	*Melania* sp.—Asia
C of *Metagonimus (takahashii)* sp.	Takahashi 1929	*Melania* sp.—Japan

C of *Cryptocotyle lingua**	Stunkard 1930	*Littorina littoria*—Mass.
*C. lophocerca**	Lebour 1911	*Paludestrina stagnalis*—England
		Littorina littoria
C. nicobaricae I	Sewell 1931	*Melanoides creber*—Camorta Isl., Nicobars
C of *Haplorchis pleurolophocerca*	Khalil 1932	*Melania tuberculata*—Egypt
C. pleurolophocerca	Sonsino 1892	*Melania tuberculata*—Tunisia
C. pleurolophocerca	Callot 1936	*Melania tuberculata*—Tunisia
C. pleurolophocerca	Langeron 1924	*Melania tuberculata*—Tunisia
C. pleurolophocerca	Brumpt 1922	*Melania tuberculata*—Gafsa, Tunisia
C of *Opisthorchis felineus (tenuicollis)*	Vogel 1934	*Bithynia leachi*—Europe
C. vogeli	Cable 1935 (1938)	*Goniobasis semicarinata*—Kentucky
C of *Apophallus venustus*	Cameron 1937	*Goniobasis livescens*—Canada
C of *Heterophyes heterophyes*	Khalil 1937	*Pironella conica*—Asia
C of *Metorchis intermedius**	Heinemann 1937	*Bithynia tentaculata*—"Kurischen Haff"
C. lophocerca	Filippi 1857, Dubois 1928	*Bithynia tentaculata*—Europe
C of *Euryhelmis monorchis*	Ameel 1938	*Pomatiopsis lapidaria*—Michigan
C of *Acanthostomum coronandum*	Rothschild 1938	*Peringia ulvae*—England
C from *Oncomelania hupensis*	Kuntz 1952	*Oncomelania hupensis*—China
C of *Haplorchid* sp.	Rothschild 1938	*Peringia ulvae*—England
C of *Centrocestus armatus*	Yamaguti 1938	*Semisulcospira libertina*—Japan
C of *Caecincola parvulus*	Lundahl 1938	*Amnicola lustrica*—Michigan
C. melanoides	Porter 1938	*Melanoides tuberculata*—Transvaal
C. britsiae	Porter 1938	*Melanoides tuberculata*—Transvaal
C. opacocorpa	Cable & Wheeler 1939	*Goniobasis semicarinata*—Kentucky
C. semicarinatae	Cable & Wheeler 1939	*Goniobasis semicarinata*—Kentucky
C. constrictovesica	Cable & Wheeler 1939	*Goniobasis semicarinata*—Kentucky
C of *Cryptocotyle jejuna*	Rothschild 1939	*Peringia ulvae*—England
C. plotiopsis	Johnston & Simpson 1939	*Plotiopsis tatei*—Australia
C of *Exorchis oviformis*	Komiya & Tajimi 1940	*Stenotyra japonica*—China, Japan
C of *Siphodera vinaledwardsii**	Cable & Hunninen 1942	*Bittium alternatum*—Mass.
C. caribbea XIII*	Cable 1956	*Bittium varium*—Puerto Rico.
C of *Metorchis conjuntus*	Cameron 1944	*Amnicola limosaporata*—Canada
C of *Telogaster opisthorchis*	MacFarlane 1945	*Potamopyrgus badia*—New Zealand
C of *Apophallus brevis*	Miller 1946	*Amnicola limosa*—Canada
C. pleurolophocercous I*	Maxon & Pequegnat 1949	*Cerithidea californica*—California

C. pleurolophocercous II*	Maxon & Pequegnat 1949	*Cerithidea californica*—California
C of *Phocitremoides ovale**	Martin 1950	*Cerithidea californica*—California
C of *Euhaplorchis californiensis**	Martin 1950	*Cerithidea californica*—California
C of *Parastictodora hancocki**	Martin 1950	*Cerithidea californica*—California
C of *Metagonimoides oregonensis*	Burns & Pratt 1953	*Goniobasis silicula*—Oregon
C of *Metagonimoides* sp.	Ingles 1953	*Goniobasis nigrina*—California
C of *Opisthorchis tonkae*	Sillman 1953	*Amnicola limosa*—Michigan
C. sigmoida	Fain 1953	*Bithynia alberti*—Lake Albert, Belg. Congo
C. atomica	Fain 1953	*Bithynia alberti*—Lake Albert, Belg. Congo
C. bithyniella	Fain 1953	*Bithynia alberti*—Lake Albert, Belg. Congo
C. orospinosa	Ullman 1954	*Melanopsis praemorsa*—Israel
C of *Stictodora tridactyla**	Martin & Kuntz 1955	*Pironella conica*—Egypt
C of *Heterophyes* sp.*	Martin & Kuntz 1955	*Pironella conica*—Egypt
*C. bermudensis**	Schell & Thomas 1955	*Batillaria minima*—Bermuda
*C. parapleurolophocercoides**	Etges 1956	*Amnicola pilsbryi*—New York
C of *Heterophyes aequalis**	Kuntz & Chandler 1956	*Pironella conica*—Egypt
C. caribbea X*	Cable 1956	*Cerithidea constata*—Puerto Rico
C. caribbea XI*	Cable 1956	*Cerithidea constata*—Puerto Rico
C. caribbea XII*	Cable 1956	*Cerithidea constata*—Puerto Rico
C. caribbea XIV*	Cable 1956	*Bittium varium*— Puerto Rico
C. caribbea XVI*	Cable 1956	*Cerithium algicola*, Puerto Rico
C. caribbea XVII*	Cable 1956	*Turritella exoleata*, Puerto Rico
C. caribbea XVIII*	Cable 1956	*Cerithium algicola*, Puerto Rico
C. caribbea XIX*	Cable 1956	*Cerithium variabile*, Puerto Rico
C of *Metadena adglobosa**	Cable 1956	*Cerithidea variabile*—Puerto Rico
C. caribbea XV*	Cable 1956	*Cerithidea variabile*—Puerto Rico
C of *Cryptocotyle concavum**	Wooton 1957	*Amnicola longinqua*— California
C of *Stellantchasmus falcatus*	Martin 1958	*Stenomelania newcombi*—Hawaii
C of *Haplorchis yokogawai*	Martin 1958	*Stenomelania newcombi*—Hawaii

*Marine or brackish water forms

C. benigna Faust 1924 (Nakagawa 1915, Cercaria sp. III; Kobayashi 1922; Cercaria 'c') BL 108-144 microns, BW 54-72 microns, OS 18 microns, EXB sac-like, tail longer than body, redia 720-900 microns long.

C. fulvopunctata Ercolani 1882 (Bregenzer 1916, *C. elegans*): BL 80-130 microns, BW "to 40 microns," TL 160 microns, Dorso-ventral fin fold. This cercaria may be the same as C of *Heterophyes aequalis.*

C of *Cryptocotyle jejuna* Rothschild 1939: CG = 2(7); CGD = 3:4::4:3; fin fold dorso-ventral, VS rudimentary.

C of *Opisthorchis tonkae* Sillman 1953—only an abstract published, which does not include the description of the cercaria.

C of *Haplorchid* fluke Rothschild 1938: CG = 2(7); CGD = 3:4::4:3.

C of *Pygidiopsis summus* Nishio 1915: CG = 2(7); CGD = 3:4::4:3.

C of *Heterophyes heterophyes* Khalil 1937: CG = 2(7); CGD = (?) 3:4:: 4:3; fin fold dorso-ventral**.

C. setaecauda Jones 1951: This C was originally described as having a stylet and without fin folds on the tail. Etges (1956, p. 89) placed this C within the parapleurolophocerca group.

C. "F", "T", "U", & "W" Miller 1925, 1929; large tailed monostome cercariae; strongly photopositive; pigmented tails.

In dealing with the cercariae whose specific name has been designated as *pleurolophocerca* the investigator may either reduce the inadequate descriptions before 1928 into synonomy with Khalil's 1928 description of C of *Kasr Aini* (*C. pleurolophocerca*); or place them as *nomen dubium.* Khalil thought these cercaria were the same as the one he later called C of *Kasr pleurolophocerca* (*Haplorchis pleurolophocerca*). Since he had material from the area of the descriptions, this writer follows his impressions and lists them as synonyms. However, Khalil's description does not indicate prominent lateral fin folds which were described by earlier workers. Langeron (1924, p. 43-44) may be right in thinking that the cercaria that he and previous workers of the area had called *C. pleurolophocerca* was identical with Sewell's (1922) description for *C. indicae* VII. This being true, there are at least two different species involved in the complex.

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**This article was in press when Martin (Tr. Am. Micr. Soc., 78: 172-180, 1959) gave the description for this species.

Some Digenetic Trematodes of Shore Birds at Friday Harbor, Washington*

HILDA LEI CHING

Reports of trematodes from migratory birds of the Pacific coast have been sparse. Park (1936) described a new heterophyid from the California gull at Dillon Beach, California. Young (1949) reported two microphallids, one new, from the stone curlew, godwit, and willet from the California coast. New host and distribution records were noted for two trematodes from the western gull at Newport, Oregon by Reish (1950). Martin (1950 a, b, c, 1951) recovered three new heterophyid genera and one new opisthorchid genus from experimental definitive hosts. The second intermediate hosts are fishes from southern California and the natural definitive hosts are probably piscivorous birds and mammals. One new notocotylid species was recovered from experimental hosts (Martin, 1956) and the natural host for it may be an aquatic bird. Martin (1955) described a new species of heterophyid trematode from the ring-billed gull in southern California.

The author collected seven species of trematodes from five birds shot in the vicinity of Friday Harbor, Washington during the summers of 1957 and 1958. All trematodes were fixed in AFA (Alcohol-Formalin-Acetic Acid) under the pressure of a cover slip except minute worms which were pipetted directly into hot killing solution. The trematodes were stored in 70 per cent alcohol, stained with Delafield's hematoxylin or Semichon's carmine, and mounted in permount.

Acknowledgments are made to Peter Stettenheim, Dr. Harvey Richardson and Harold Rogers for collections and identifications of the birds; to the staff at the Friday Harbor Laboratories of the University of Washington for use of laboratory facilities; to Dr. H. W. Manter for supervision of the work. Studies at the Friday Harbor Laboratories were made possible by summer grants from the National Science Foundation (1957) and the Wolcott Memorial Fund of the University of Nebraska (1958).

Type specimens of all new species are deposited in the Helminthological Collection of the U. S. Museum. Measurements are in millimeters unless specified otherwise.

FAMILY HETEROPHYIDAE

1. *Galactosomum humbargari* Park, 1936

HOSTS: *Larus heermanni* Cassin, Heermann's gull
Larus glaucescens Naumann, glaucous-winged gull
Larus philadelphia (Ord), Bonaparte's gull

HABITAT: Intestine

Four to ten specimens were found in each of the above hosts. The birds were examined several hours after their death and some of the specimens were somewhat macerated, although still identifiable. Park (1936) described *G. humbargari* from *Larus californicus* at Dillion Beach, California. This report is a new host and locality record.

2. *Cryptocottyle lingua* (Creplin, 1825) Fischoeder, 1903

HOST: *Larus glaucescens* Naumann, glaucous-winged gull

HABITAT: Intestine

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One specimen of this heterophyid was collected. It appeared to be average in body size and internal organs.

Stunkard and Willey (1929) and Stunkard (1930) have studied the life cycle of *C. lingua* in detail. The pleurolophocercous cercaria develops in *Littorina littorea,* penetrates and encysts in the cunner, and excysts in the gut of birds and mammals, the definitive hosts. A similar larval trematode was found by the author in 2.4% of 2,140 *L. sitkana* and *L. scutulata* examined. The cercaria readily encysts on the fins and tail of tide pool sculpins. Further experimental work is needed to determine whether this particular cercaria is that of *C. lingua.*

PHILOPHTHALMIDAE

3. *Echinostephilla haematopi* n. sp. (Figs. 1-4)

HOST: *Haematopus bachmani* Audubon, black oystercatcher

HABITAT: Lower part of intestine

LOCALITY: Goose Island

Fifteen specimens were obtained in the summer of 1957. The trematodes were alive but most of the scales had been lost in all but three specimens. The following summer, approximately 50 worms were collected from another bird and these were examined carefully when alive.

The trematodes have an orange-red appearance when living and are usually curled ventrally into a C-shape. Longitudinal muscles are strongly developed throughout the body.

DESCRIPTION (measurements on 10 specimens, average in parentheses): Body elongate, thick, with tapering anterior end and rounded posterior end, length 3.4 to 6.8 (5.14); width at acetabular level 1.1 to 1.3 (1.22). Body scales arranged in quincunx fashion at the anterior end; each scale embedded in a fold of cuticula. Scales disappear at varying levels posterior to acetabulum. Extreme anterior end of body scaleless. A short space separates a double row of dorsal scales near the anterior end from the other body scales (Fig. 3). These two alternating rows terminate on the ventral surface with three to four scales on each side. These "head spines" probably represent the collar spines of echinostomes and a few philophthalmids. The largest "head spines", 10 to 12 microns in length, are equal in size to the body scales of the eighth row.

Oral sucker round, subterminal, 0.17 to 0.23 (0.20); acetabulum one-third to one-fifth of body length from anterior end, round, about three times the size of oral sucker, 0.49 to 0.64 (0.58). Prepharynx almost lacking to 0.31 (0.14) in length. Pharynx oval, 0.11 to 0.14 by 0.06 to 0.12 (0.12 by 0.10). Esophagus two to three times length of pharynx, 0.22 to 0.47 (0.36), bifurcates one-third to one-fourth from anterior end of body. Intestinal ceca long and narrow, terminating blindly at posterior end of body, lined with cuboidal cells. Testes round to subglobular, oblique to tandem, touching each other, located between ends of ceca, 0.18 to 0.35 by 0.32 to 0.43 (0.24 by 0.39).

Genital pore median, closely anterior to acetabulum. Cirrus sac of two portions separated by a constriction approximately 0.1 long (Fig. 2). Anterior portion club-shaped, extending 0.05 to 0.4 (0.2) posterior to acetabulum, surrounded by a thin membrane, contains cirrus, and pars prostatica.

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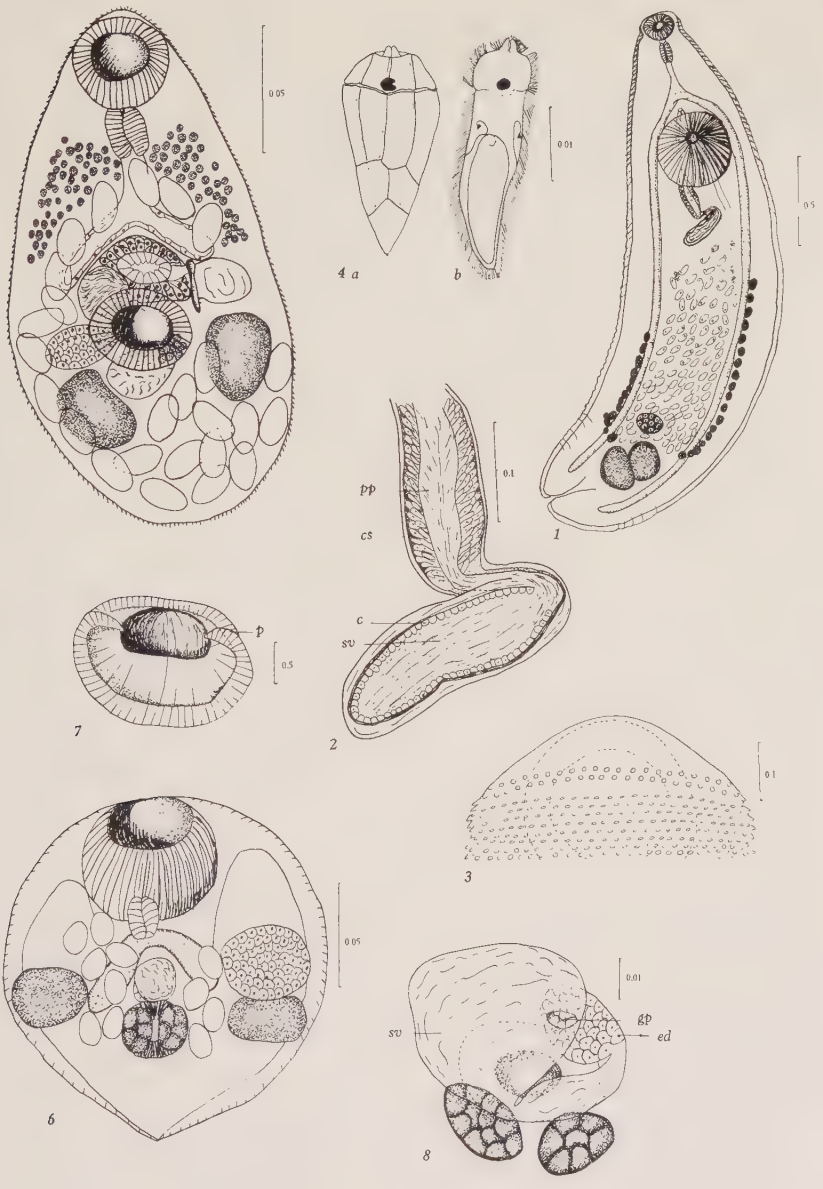
c = cell lining; cs = cirrus sac; ed = ejaculatory duct; gp = genital pore; p = papilla of oral sucker; pp = pars prostatica; sv = seminal vesicle.

All figures were drawn with the aid of a camera lucida. Scales projected indicate the value in millimeters.



Figures 1-4. *Echinostephilla haematopi* n. sp.
Figure 1. Ventral view; Figure 2. Portion of cirrus sac; Figure 3. Dorsal view of anterior end; Figure 4. Miracidium. a. View showing epithelial plates, b. View of internal anatomy.

Figure 5. *Plenosoma minimum* n. gen., n. sp., ventral view.

Figures 6-8. *Gymnophallus obscurus* n. sp.
Figure 6. Dorsal view; Figure 7. Oral sucker, ventral view; Figure 8. Terminal genitalia, dorsal view.

Cirrus protrusible, finely spined, opening into the genital pore posterior to metraterm. Duct between cirrus and pars prostatica short, nonglandular, unspined. Pars prostatica surrounded by numerous prostatic cells in posterior part of anterior portion of cirrus sac. Posterior portion of cirrus sac elongate, 0.25 to 0.50 by 0.07 to 0.17 (0.38 by 0.13), muscular with overlying membrane 6 to 12 microns thick, contains slightly coiled or straight seminal vesicle. Seminal vesicle lined with single layer of cuboidal cells.

Ovary smaller than testes, oval, 0.11 to 0.18 by 0.20 to 0.28 (0.15 by 0.25), prestesticular and medianly located. Laurer's canal not observed. Receptaculum seminis uterinum present. Uterus intercecal, slightly overlapping anterior testis posteriorly, winding anteriorly, becoming muscular at level of seminal vesicle. Vitellaria mostly lateral to ceca, ranging in number from 10 to 35 on each side, usually with a lesser number on the right side; vitelloducts join posterior to ovary to form small yolk reservoir. Eggs oval, 50 to 74 by 32 to 37 microns; embryonated eggs larger at one end. Miracidia with well developed eye spots, lie with anterior end at the larger (operculated) end of egg, hatch and become free in the anterior part of uterus.

Excretory pore large, opening terminally or subterminally. Excretory vesicle Y-shaped; the two lateral branches reach anteriorly to level of pharynx, then tubules pass posteriorly. Flame cells too numerous to determine a formula; tufts of long cilia are situated in the lateral tubules at regular intervals.

DISCUSSION: *E. haematopi* differs from the type species, *E. virgula* Lebour, 1909, in that the anterior end is tapered, it lacks scales on the posterior third of the body, the ovary is smaller than the testes, and it has fewer (10-35) vitellarian follicles than 40 to 60 on each side.

Shelswell (1954) redescribed *E. virgula* and found the crown of "head spines" was incomplete ventrally, the egg sizes varied greatly in fixed and living specimens, and "extra and intra-cirral seminal vesicles" were present. The latter term "extra-cirral seminal vesicle" may mean that this organ is outside the cirrus sac proper. However, it may also imply a seminal vesicle lying free in the parenchyma.

The seminal vesicle in specimens of *E. haematopi* is enclosed within the cirrus sac and separted from the anterior portion of the cirrus sac by a narrow constriction. This condition is probably true for *E. virgula;* the "intra-cirral seminal vesicle" refers to the pars prostatica in the anterior portion of the cirrus sac, and the "extra-cirral seminal vesicle" refers to the posterior portion of the cirrus sac containing the seminal vesicle as in my description of *E. haematopi.* The thick wall and underlying muscles of the posterior portion of the cirrus sac and the cellular lining of the seminal vesicle may be generic characteristics that have not yet been described.

Echinostephilla was formerly considered to be an isolated genus in the family Echinostomatidae. Shelswell placed it tentatively in the family Philophthalmidae with those species having small pharynges, inconspicuous collars, and body spines. It is probably closely related to the echinostomes but the muscular body, sparse vitellaria, and oculate miracidia which hatch in utero clearly place it in the family Philophthalmidae.

Skrjabinovermis Belopol'skaya, 1953 of the family Philophthalmidae was erected for the species *S. vesiculata* found in the ruddy turnstone, *Arenaria interpres.* This bird is also the host for *E. virgula.* The *Zoological Record* of 1953 states that *Skrjabinovermis* is "clearly a synonym of *Echinostephilla* Lebour, 1909." The similarity of figures by Shelswell (1954, p. 129) and

Belopol'skaya (1953, p. 54) and the descriptions of the two is very great. However, the description of *S. vesiculata* does not mention the presence of two rows of "head spines" and the seminal vesicle is said to lie free in the parenchyma. "Head spines" may be easily overlooked in preserved specimens and the figure does indicate a membrane around the seminal vesicle which is continuous with the cirrus sac. Because of the similarity of hosts, body shape, and measurements of body, organs, and eggs, *S. vesiculata* is considered a synonym of *E. virgula*.

In *Helminthological Abstracts of 1953* (996i), appears the statement that *Skrjabinovermis* "resembles *Cloacitrema* in possessing a seminal vesicle, which is enclosed in the genital bursa, and a seminal receptacle." The abstracter inadvertently described the placement of the seminal vesicle *within* the genital bursa which the author believes is the true condition. However, the actual translation of Belopol'skaya (1953, p. 55) reads: "The newly described genus *Skrjabinovermis* is closest to the genus *Cloacitrema* Yamaguti, 1935 in that representatives of these two genera have a seminal vesicle not enclosed in a genital bursa, and a seminal receptacle in uterinum is present." The resemblance of *S. vesiculata* to *E. virgula* which appears to have a seminal vesicle within the cirrus sac, and the figure of *S. vesiculata* as previously mentioned are reasons to assume that the seminal vesicle is really enclosed within the cirrus sac.

NOTES ON LIFE HISTORY OF *Echinostephilla haematopi*

MIRACIDIUM. Miracidia were freed from several specimens and exposed to common high tide-pool gastropods such as *Acmaea digitalis* Eschsholtz, *A. persona* Eschsholtz, *Littorina scutulata* Gould, *L. sitkana* Philippi and *Thais lamellosa* (Gmelin). The miracidia swam about erratically and one appeared to penetrate the mantle of *L. scutulata*. However, examination of the digestive glands and intestine of all these snails after one month revealed no trematodes.

The morphology of the miracidium appears to be similar to that of *Parorchis acanthus* as described by Rees (1940). The miracidium is light-sensitive with median eyespots located at the anterior end (Fig. 4b). A single motionless redia fills the posterior half. No other detail except the faint oral sucker could be determined in the redia. Anterolateral to the redia is a flame cell. A typical miracidium measured 33 by 11 μ when alive. The redia within was 18 by 9 μ. The outer surface of the miracidium is covered with abundant cilia which beat differently around the anterior and posterior parts and the mouth. The epithelial plates number six, seven, three and two in each tier from the anterior end. (Fig. 4a).

JUVENILE STAGE. Also found in the intestine of the oyster-catcher were very young trematodes possessing body spines arranged in quincunx order and an incomplete collar consisting of two alternating rows of scale-like spines. These may be juvenile stages of *E. haematopi* which the host recently ingested. No forms intermediate between these and the adults were found. A typical specimen had the following measurements: length 0.37; width 0.13; oral sucker and acetabulum equal in size 0.058; prepharynx 0.012; pharynx, 0.026 by 0.017; esophagus 0.043. The head spines are twice as long as the body spines and arranged in the same alternate fashion as in the adults. The acetabulum is located in the middle of the body. The excretory system was similar to that of the adult. In life, very small gland cells were seen. These were arranged in two rows of V-shaped assemblies anterior to the acetabulum.

The stomach of the bird contained limpets of the species *Acmaea digitalis*. Cercariae of *E. haematopi* may encyst in these limpets and infect birds in this manner. However, examination of limpets near the laboratories failed to show such an infection. Limpets on Goose Island were not examined.

Microphallidae

4. *Levinseniella propinqua* Jägerskiold, 1907

Four specimens were found in the intestine of *Haematopus bachmani* Audubon, black oystercatcher. These fit the description and measurements of specimens found on the west coast of Sweden in *Haematopus ostralegus* and *Charadrius hiaticula*. The distinctive feature of this species is the large male copulatory organ containing four thimble-like pockets, each with three-pointed hook-like structures. This report is a new host and a new locality record.

5. *Microphallus primas* (Jägerskiold, 1909) Stunkard, 1951

Stunkard (1951) transferred this species from *Spelophallus* Jägerskiold, 1909 to *Microphallus* Ward, 1901 and Belopol'skaya (1952) transferred it to *Spelotrema* Jägerskiold, 1901.

Eight microphallids fitting the description of *M. primas* were recovered from the intestine of *Haematopus bachmani* Audubon, black oystercatcher. All except one were fixed in the natural position, the body showing a ventral concavity. Total measurements were difficult to make because of this condition. In general, however, the trematodes lay within the minimum size range given by Jägerskiold (1909). The distinctive feature of the genus, that of a muscular female pore opening superficially near the genital atrium was clearly evident. Jägerskiold collected several specimens of *M. primas* from the European oystercatcher, *H. ostralegus*, but based his description on material collected from the eider duck. He did mention that the oral and ventral suckers in the trematodes of the oystercatcher were equal in size while those from the eider duck had a ratio of 1:1.4 to 1.5. The first condition is true for my specimens. Also, the testes are as large or larger than the ovary; quite the opposite condition existed in Jägerskiold's specimens. This size difference may be due to the age of the specimens, mine being barely mature. The other difference from the description is the larger genital papilla. The papilla shown in Jägerskiold's figure is one-half the size of the oral sucker. In six of my specimens, it is as large or slightly larger than the oral sucker which measures 0.043 to 0.058.

The differences from the original description appear to be the equal size of the suckers, the larger genital papilla and possible the larger testes. Since the specimens are not quite mature, they are tentatively placed in this species.

6. *Plenosoma minimum* n. gen., n. sp. (Fig. 5)

Host: *Haematopus bachmani* Audubon, black oystercatcher

Habitat: Intestine

Locality: Goose Island

Description (based on ten specimens, average in parentheses): Body pyriform, spined, very small; length 0.180 to 0.260 (0.229); width at acetabular level 0.120 to 0.150 (0.130). Oral sucker round, 0.035 to 0.049 (0.044); mouth subterminal. Acetabulum same shape and size as oral sucker, immediately post-equatorial. Prepharynx very short in most specimens to 0.020 in

one. Pharynx round to oval, 0.017 to 0.025 by 0.014 to 0.019 (0.017 by 0.014). Esophagus variable, often obscured by the uterus; in five specimens the esophagus was lacking or up to 0.017. Ceca short, extending to midbody, lined with a single layer of cells. Entire digestive system lies in anterior half of body. Genital opening a longitudinal slit to the left and antero-lateral to acetabulum. Genital atrium oval to round, 0.017 to 0.029 (0.025) with muscular folded walls. Cirrus sac lying transversely anterior to and partly overlapping acetabulum, containing cirrus, prostatic vesicle and cells, and seminal vesicle; cirrus enters anterior part of genital opening. Seminal vesicle globular, posterior portion lying dorsal to acetabulum. Prostatic cells numerous. Pars prostatica well developed with cells that taper sharply within the vesicle; vesicle 0.017 to 0.032 by 0.012 to 0.021 (0.023 by 0.014). Testes ovoid, smaller or equal to acetabulum, their anterior levels overlapping acetabular level, widely separated laterally, slightly oblique in posterior half of body. Ovary oval to pyriform, located on the right of acetabulum overlapping it dorsally. Seminal receptacle pear-shaped, extending over posterior edge of acetabulum dorsally and posteriorly, approximately same size as ovary. Yolk reservoir with distinct follicles, superimposed dorsally on anterior part of seminal receptacle. Laurer's canal not observed. Vitelline follicles distributed lateral to digestive system reaching the level of pharynx, confluent dorsally at level of esophagus, not extending past ceca. Uterus fills hindbody, extends anteriorly on right side of acetabulum to form anterior transverse loop at level of pharynx. Eggs relatively large, not more than 100 per worm (average 50), 17 to 27 by 10 to 14 microns (22 by 12 microns). Excretory bladder V-shaped with rounded lobes reaching the level of the testes. Excretory pore terminal.

The genus name *"Plenosoma"* means full body; *"minimum"* refers to the small size of the body.

DISCUSSION: *Plenosoma* resembles *Pseudospelotrema* Yamaguti, 1939 and *Maritreminoides* Yoshida, 1938 in the extent of the uterus into the forebody. However, it differs from these genera in the extent of the vitellaria, and the presence of a large muscular genital atrium and a longitudinal genital opening. In *Pseudospelotrema,* the vitallaria are marginal at the ovariotesticular level. In *Maritreminoides,* the vitellaria are largely anterior and lateral to the acetabulum. In *Plenosoma,* the vitellaria commence at the posterior level of the ceca, reach the level of the pharynx, and are lateral to the digestive system. The small, distinct follicles which are confluent dorsally at the esophagus are characteristic of the species.

Another genus with vitellaria located more anteriorly than usual in the microphallids is *Pseudosellacotyla* Yamaguti, 1954. The vitellaria are clustered symmetrically at the level of the esophagus and ceca. The type and only species, *P. lutzi* (Freitas, 1941) Yamaguti, 1954 differs fom *P. minimum* in having no cirrus sac, a bipartite seminal vesicle and a genital pore opening ventral to the acetabulum.

The presence of a muscular genital atrium recalls the complicated male copulatory organ of *Levinseniella* Stiles and Hassall, 1902. The atrium in *P. minimum* is much simpler, containing no male papilla or thimble-shaped pockets. *Levinseniella* does not have a cirrus sac.

GENERIC DIAGNOSIS OF PLENOSOMA

Very small, spined-microphallids with pyriform shape. Oral sucker and acetabulum round, of equal size, the latter postequatorial. Digestive system in anterior half of body. Prepharynx short, pharynx round to oval, esophagus

short, ceca reaching only to genital atrium. Genital opening a longitudinal slit to the left of acetabulum. Genital atrium large and muscular, with no distinct pockets within. Cirrus sac with cirrus, prostate cells, pars prostatica, seminal vesicle; extending transversely anterior to acetabulum. Testes oval, far apart, subsymmetrical, in posterior half of body. Ovary oval, on right of acetabulum partly overlapping it dorsally. Seminal receptacle and yolk reservoir present. Laurer's canal not observed, probably present. Uterus with coils anterior and posterior to acetabulum, extends into forebody. Eggs large but not numerous, often filling most of the body. Vitellaria consist of small distinct follicles lateral to the digestive system and extending from pharynx to level of ceca. Excretory bladder V-shaped, excretory pore terminal.

TYPE SPECIES: *P. minimum.*

7. *Gymnophallus obscurus* n. sp. (Figs. 6-8)

HOST: *Haematopus bachmani* Audubon, black oystercatcher

HABITAT: Intestine

LOCALITY: Goose Island

FREQUENCY: More than 100 in one host

DESCRIPTION (measurements on ten specimens, average in parentheses): Body almost round to pyriform with narrow posterior end, length 0.2 to 0.3 (0.23); width 0.17 to 0.20 (0.18). Spines in transverse rows covering the body. Oral sucker twice the diameter of acetabulum, terminal to subterminal, 0.072 to 0.106 (0.086); some specimens showing lateral papillae on ventral surface of oral sucker (Fig. 7). Acetabulum 0.035 to 0.047 (0.038) in diameter, usually located in anterior part of posterior half of body; in contracted specimens it is mid-equatorial. Pharynx round, 0.014 to 0.021 (0.018), the main portion located dorsal to oral sucker. Esophagus length variable; ceca short, in anterior half of body.

Genital pore slightly to the right, immediately anterior to acetabulum. Testes ovoid, subsymmetrical, slightly anterior or slightly posterior to acetabulum. Cirrus sac lacking. Seminal vesicle large and ovoid, dorsal to acetabulum or directly anterior to acetabulum. Ejaculatory duct with large glandular cells, entering genital pore anteriorly (Fig. 8). Ovary ovoid, larger than testes, far to the right and anterior to acetabulum. Seminal receptacle and Laurer's canal not observed. Vitellaria consist of two compact oval masses dorsal to acetabulum or overlapping acetabulum slightly posteriorly or slightly anteriorly. Each mass measures 0.017 to 0.029 (0.023) in length. Uterus may fill most of body, extending to posterior edge of oral sucker. Eggs oval, 14 to 15 by 8 to 10 microns.

Excretory bladder V-shaped with lateral branches reaching to oral sucker.

The species name *"obscurus"* indicates that the worm is inconspicuous in body size and internal organs.

DISCUSSION: The subfamily Gymnophallinae has been included in the family Microphallidae since 1924. Cable (1953) transferred this subfamily to the Fellodistomatidae on the basis of life cycle experiments on *Parvatrema borinquenae* Cable, 1953. Morosov (1955) erected a new family, Gymnophallidae. Yamaguti (1958) still included Gymnophallinae in Microphallidae and made a new subfamily, Parvatrematinae, to include *P. borinquenae.*

The combination of small size, large excretory bladder, and numerous eggs often makes species of *Gymnophallus* difficult to study. Early descriptions were not complete and specific identification is so uncertain that Stunkard and Uzmann (1958) declined to name three *Gymnophallus* sp. recovered from

wild ducks. However, they did name a new species for their fourth gymnophallid, *Parvatrema borealis,* which differs from *P. borinquenae* in geographical distribution and primary and secondary hosts. They transferred *Gymnophallus ovoplenus* Jameson and Nicoll, 1913 to the genus *Parvatrema* even though the morphology of that species is imperfectly known and no figure was given in the original description.

In *G. obscurus,* the shape of the body and location of the vitellaria, acetabulum, and gonads appear to vary with the amount of body contraction, the extension of the excretory bladder, and the number of eggs. The shapes and sizes of the gonads, vitellaria, and eggs remain constant. The arrangement of the compact vitellaria and gonads is like that in *Gymnophallus macroporus* Jameson and Nicoll, 1913. However, the body and eggs in *G. macroporus* are twice the size of *G. obscurus* and the uterus does not extend anterior to the intestinal ceca as it does in *G. obscurus.* The arrangement and shape of the vitellaria, the body and egg sizes correspond to that of *P. borealis* but the genital pore of *G. obscurus* is immediately anterior to the acetabulum rather than being some distance anterior to the acetabulum.

More information is needed on the morphology, host specificity, and size differences of gymnophallids.

SUMMARY

One new genus, *Plenosoma* (Microphallidae), and three new species, *Plenosoma minimum* (Microphallidae), *Gymnophallus obscurus* (Microphillidae) and *Echinostephilla haematopi* (Philophthalmidae) are described from the intestine of *Haematopus bachmani* Audubon. New host and locality records are noted: *Galactosomum humbargari* Park 1936 from *Larus heermanni* Cassin, *L. glaucescens* Naumann, and *L. philadelphia* (Ord); *Cryptocotyle lingua* (Creplin, 1825) Fischoeder, 1903 from *Larus glaucescens; Levinseniella propinqua* Jägerskiold, 1907 and *Microphallus primas* (Jägerskiold, 1909) Stunkard, 1951 from *Haematopus bachmani* Audubon.

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An Experiment on the Combined Pathogenic Effects of *Haemonchus contortus* and *Nematodirus spathiger* on Lambs

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Various workers have demonstrated that pure infections of *Haemonchus contortus,* an abomasal nematode, produce a severe, often fatal, anemia in sheep; much of the work on the pathogenesis of this parasite has been summarized by Andrews (1942) and Lucker (1952). Diarrhea and marked anorexia and loss in condition are not usually associated with uncomplicated haemonchosis. It has also been demonstrated experimentally by the writers (1953a) and by Seghetti and Senger (1958) that massive, pure infections of *Nematodirus spathiger,* an intestinal nematode, produced an enteritis in lambs accompanied by diarrhea, anorexia, and retardation in growth, but a marked anemia was not associated with pure infections of this parasite. Recently, Kingsbury (1953), Thomas and Stevens (1956), and Baxter (1957) have reported outbreaks of nematodirosis in lambs in Great Britain caused by other species of *Nematodirus,* namely, *N. battus* and *N. filicollis.* The clinical symptoms displayed by the affected animals in these outbreaks were similar to those reported by the writers (*loc. cit.*) and by Seghetti and Senger (*loc. cit.*). Furthermore, Turner and Colglazier (1954) and Colglazier and Turner (1955) reported data suggesting that clinical infections of *H. contortus* may alter the normally self-limiting course of infections of *N. spathiger* in lambs when these infections were acquired on pasture.

As a consequence of these latter studies, which had been carried out and reported in abstract earlier (*vid.* Turner and Colglazier, 1954), it seemed desirable to investigate with a small-scale, controlled experiment the effects of simultaneous infections of *N. spathiger* and *H. contortus* on lambs. The results of this experiment were previously reported in part in abstract by the writers (1953c).

MATERIALS AND METHODS

Four female and four wether pure-bred Shropshire lambs, 66 to 90 days old and weighing 27 to 45 pounds at the beginning of the experiment, were used. Four of these lambs were infected experimentally and four served as controls. Each infected lamb was paired with an uninfected control lamb of about the same weight. Two pairs of infected and control lambs were in the high weight range and two pairs in the low weight range. The numbers and initial weights of the lambs are noted in Figure 1A.

The infective larvae were obtained from standard charcoal-feces cultures. The four lambs to be infected (Nos. 352, 342, 353, and 348) were each given 18,000 infective larvae of *H. contortus per os*. Two of these four lambs (Nos. 352, 342) were also infected with *N. spathiger* in a similar manner as follows: A dose of 100,000 larvae were given each simultaneously with the dose of *H. contortus* larvae; three doses of 50,000 larvae each were given semiweekly

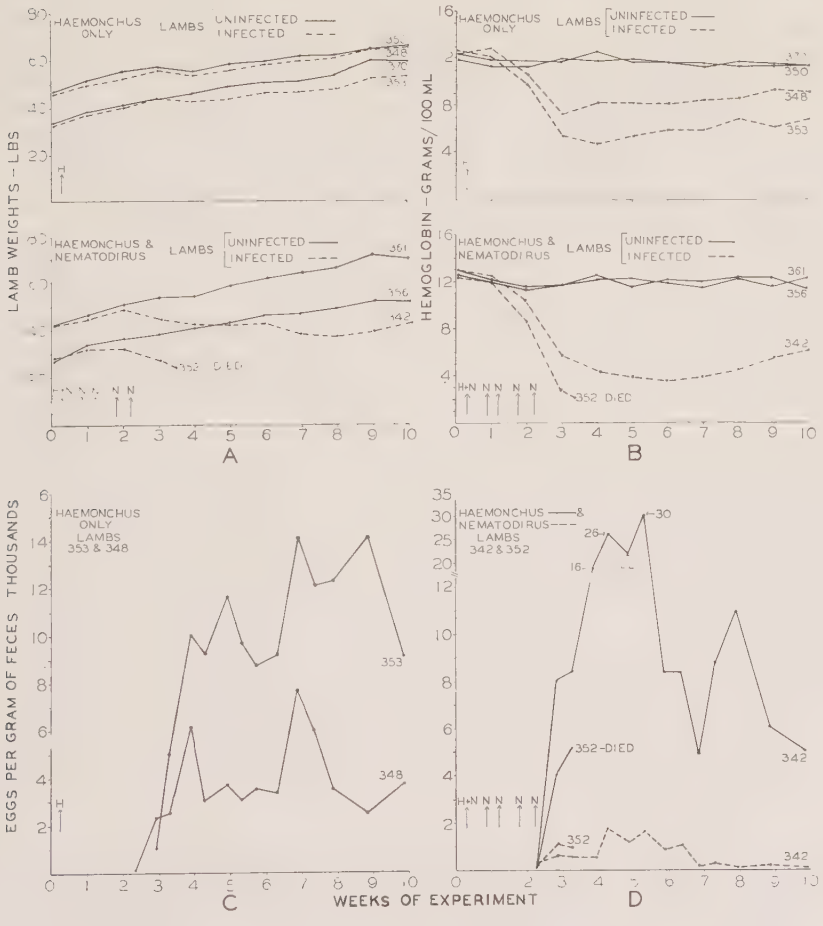

Fig. 1. Weight data (A), hemoglobin levels (B) of infected and control lambs, and fecal parasite egg counts (C, D) of the infected lambs. Arrows indicate when infective larvae were administered to the lambs; see text for quantities of larvae given.

thereafter, and a final dose of 40,000 larvae was given each of these lambs early in the third week after the initial infection, which made a total of 290,000. Figure 1 indicates precisely when these larvae were administered. On the basis of previous experience with experimental infections with these parasites in lambs of similar age and breed, these larval doses of each species were considered to be in the sublethal range. However, whether or not a dose of larvae is lethal is subject to wide variation (*vid.* Lucker, 1952; the writers, 1953a; Seghetti and Senger, 1958). This dosage schedule employed to infect the lambs with *N. spathiger* was chosen to simulate as closely as possible the conditions of infection of lambs with this parasite of Group B, Experiment 2, of the writers' previous experiments on pure infections (1953a). Because of limited availability of *N. spathiger* larvae at the time the present experiment was initiated, it was not possible to infect a third pair of lambs with only *N. spathiger* simultaneously with the others, but it was believed that the writers' (*loc. cit.*) previous experiment represented closely the situation involving pure *N. spathiger* infection in similar lambs maintained under comparable conditions.

During the course of the experiment the lambs were housed in concrete-floored pens with outside runways. The pair of lambs with infections of both nematode species were kept in one pen, those with only *H. contortus* in another, and the uninfected controls in a third pen. Otherwise, the lambs were fed and the course of their infections studied in the same manner as those of Group B, Experiment 2, of the writers' previous work on pure infections of *N. spathiger* (*loc. cit.*)

Results and Discussion

The principal results of this experiment are summarized in Figure 1 and Table 1. Substantial infections with *H. contortus* were established in all four lambs, but more worms of this species appeared to have become established in the lambs infected with both species than in those infected with only *H. contortus,* and more of this species apparently became established in the lightest lamb of each pair than in the heaviest. The fact, however, that lamb 352 died 23 days after infection, whereas the others were not examined for worms until 10 weeks after infection, gave less opportunity for this lamb to lose spontaneously some of its infection. Of the two lambs given only larvae of *H. contortus,* both of which survived the 10-week experimental period, the smaller lamb (353) had the higher fecal parasite egg count (Fig. 1C), and the larger number of worms at necropsy (Table 1). The infections of *N. spathiger* established in lambs 352 and 342, which were also infected with *H. contortus,* appeared from the fecal egg count data to follow the same pattern as pure infections of lambs with *N. spathiger* previously reported by the writers (1953a). Although lamb 352 died 23 days after infection, the fecal egg counts of *N. spathiger* were rising in a normal fashion at the time of death (Fig. 1D). *Nematodirus* egg counts of lamb 342 followed the course of such counts obtained from the writers' previous pure infections (*loc. cit.*), with the exception that high egg counts continued about a week longer than in pure infections with this parasite.

The most significant feature of the results of this experiment was the apparent enhancement of the typical clinical effects of haemonchosis and nematodirosis in lambs infected with both species of nematodes. These effects were compared with those observed in lambs infected only with *H. contortus* in this experiment and those infected only with *N. spathiger* in the previous experiment described by the writers (1953a). The pure *Haemonchus* infections

established in lambs 348 and 353 had only a very slight depressant effect on growth (Fig. 1A) and feed consumption, and these lambs were in excellent physical condition when the experiment was terminated. However, the growth of lambs 342 and 352 with dual-species infections was markedly depressed. At death 23 days after infection, lamb 352 weighed 3 pounds less than at the start of the experiment, whereas its control had gained 12 pounds. The weight differential between dually infected lamb 342 and its uninfected control (361) 10 weeks after infection was 26½ pounds. This depression in weight gain of the lambs with both species of nematodes was much greater than had occurred in comparable lambs with similar pure infections of one or the other of the two species.

The lambs with dual-species infections also developed a more acute anemia than that which occurred in the lambs with pure *Haemonchus* infections (Fig. 1B). Lamb 352, which died, had a terminal hemoglobin level of 2.2 gr. per 100 ml of blood and a terminal hematocrit level of 6 per cent. The hemoglobin and hematocrit levels of lamb 342 reached near fatal levels of 3.7 gr. and 13 per cent, respectively, 6 weeks after infection, and gradually improved thereafter. The lowest hemoglobin and hematocrit levels of the lambs with pure *Haemonchus* infections were 4.7 gr. and 15 per cent, respectively, for lamb 353, the lighter of the pair. The fact that the anemia was most acute in the lambs infected with both species of nematodes was probably related to the marked reduction in feed consumption of these lambs, which probably had an adverse effect upon hematopoiesis.

Between the third and fourth weeks after infection the total daily feed consumption of lambs 342 and 352 was reduced to ½ pound or less per lamb per day, whereas the feed consumption of lambs 348 and 353, which were infected with *Haemonchus* only, during the same period was only slightly reduced compared with the controls which were eating in excess of 3 pounds of hay per lamb per day. Therefore, the depression in appetite of the lambs with dual-species infections was primarily, but not entirely, due to the *Nematodirus* infections.

Another important clinical feature of the mixed infections of the two species in the lambs was the occurrence and intensity of diarrhea. The two lambs with pure infections of *Haemonchus contortus* had feces of normal consistency throughout the experiment, whereas lambs 352 and 342 with mixed infections developed acute diarrhea, which began on the 12th and 13th day after infection, respectively, and continued for several days. The feces of lamb 352 returned to normal consistency before it died 23 days after infection. However, lamb 342 had mushy feces almost continuously after the acute phase of diarrhea had passed which continued up to the termination of the experiment. The diarrhea in these lambs was more acute and of longer duration than that observed by the writers in similar lambs with comparable exposure to pure infections of *N. spathiger* (1953a). Furthermore, the diarrheic feces of the

Table 1. Nematodes Recovered at Necropsy.

Nematodes	Infection and Lamb Numbers			
	Both Species		*Haemonchus* Only	
	352*	342	353	348
H. contortus	6,130	2,580	3,020	980
N. spathiger	75,000	14,000		

*Died 23 days after infection; other lambs necropsied 10 weeks after infection.

lambs with dual-species infections were dark brown, or almost black, in color, whereas diarrheic feces of lambs with pure infections of *N. spathiger* were light brown. The blackish color of these diarrheic feces was undoubtedly due to the presence of excessive amounts of blood, or rather the decomposition products of the contained hemoglobin. Andrews (1939) also observed that the fluid stools in experimental cases of trichostrongylosis in sheep and goats were light brown in color and free of blood in pure infections. He commented that the so-called "black scours," which various authors have reported as characteristic of naturally acquired trichostrongylosis, may have been due to concurrent haemonchosis or possibly green forage. It has also been noted by the writers (1953b and unpublished) that fluid stools of clinically affected lambs, which were experimentally infected with only *T. axei, T. colubriformis,* or *N. spathiger,* or mixed infections of the latter two species, were light brown in color, even when the feed used was good grade alfalfa hay with a high green leaf content. Therefore, it is somewhat misleading that "black scours" and trichostrongylosis have been used synonymously in the literature. "Black scours," however, may be a useful nontechnical term to apply to cases of trichostrongylosis, nematodirosis, or other parasitic infections which are accompanied by acute diarrhea, when concomitant haemonchosis, hookworm, or coccidial infections are present and responsible for significant loss of blood from the gut.

It may be concluded from the data presented herein and that reported by Turner and Colglazier (1954) and Colglazier and Turner (1955) that the clinical effects of *N. spathiger* and *H. contortus,* when significant numbers of these two parasites are simultaneously present in lambs, are additive. Also, the characteristic symptoms that are generally associated with each species are usually enhanced. Furthermore, when mixed infections of these two parasites occur in lambs, there appears to be little antagonistic action between them that causes partial or complete elimination of one or the other species. In this regard, attention should be called to the fact that Kates *et al.* (1957) reported that when infections of three species of stomach worms (*T. axei, Ostertagia circumcincta,* and *Haemonchus contortus*) were simultaneously induced in lambs, the clinical effects of each species were not simply additive. Instead, these species interacted antagonistically, as was shown by the fact that the infections of *H. contortus* were almost completely eliminated and those of *O. circumcincta* were partially eliminated.

SUMMARY

Eight Shropshire lambs were used in a small-scale experiment to determine the combined effects of *Haemonchus contortus* and *Nematodirus spathiger* in lambs that were otherwise free of parasites. Four lambs were kept as controls and four were infected—two with both species of nematodes and two with *H. contortus* only. Observations made during the 10 weeks of the experiment showed that the combined effects of these two parasitic nematodes were additive, and that the characteristic symptoms of haemonchosis and nematodirosis were generally accentuated.

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The Histopathology of *Crepidostomum* sp. Infection in the Second Intermediate Host, *Sphaerium striatinum**

THOMAS C. CHENG and HUGO A. JAMES

The life history of *Crepidostomum cornutum* (Osborn, 1903) was originally described by Ameel (1937) and later verified by Cheng (1957) who included the description of the metacercarial stage which is encysted in the cardiac region of crayfishes. In Sinking Creek, Giles County, Virginia, the crayfish intermediate host is *Cambarus bartoni sciotensis* Rhodes. During the summer of 1958 the authors, while working with other helminths known to be present in Sinking Creek, were amazed at the number of empty bivalve shells identified as *Sphaerium striatinum*, the first intermediate host of *C. cornutum*. A concerted effort resulted in the collection of over 100 living specimens of this bivalve from a 100 foot segment of the creek, under and on each side of the covered bridge on the Pembroke Road. In addition to these specimens, hundreds of empty shells were found. The other molluscs in the same area were seemingly healthy.

OBSERVATIONS

A few of the bivalves were dissected in the laboratory and all were found to be infected with first and second generation rediae of *C. cornutum*. The remaining clams were placed in distilled water and within 12 hours most were releasing cercariae. Twenty five clams which were not shedding cercariae were dissected without revealing any evidence of infection. All were gravid females. All of the males examined were heavily infected. The mother rediae

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are located on the gills while the daughter rediae are in the hepatopancreas. No conclusive explanation can be offered at this time for the observed sex difference; perhaps there is some hormonal action in gravid females which brings about a resistance to further infection.

Infected clams were sectioned at 10 microns and stained with Mallory's Triple Connective Tissue Stain. The hepatic mass was almost completely filled with second generation rediae in varying degrees of maturity. Some enclosed active cercariae while others included germ balls at various stages of metamorphosis (Cheng and James, In Press). The diameters of the daughter rediae were from 1.5 mm. to 4 mm. and the average hepatic mass enclosed 35 to 45 individual rediae in addition to cercariae which had escaped from ruptured rediae. The liver cells had practically all disappeared, but a few isolated cells were seen. Traces of bile ducts are visible in a few specimens. The collagenous fibers which surround the hepatopancreas remained almost completely intact as did the single layer of epitheliam external to the collagenic tissue. In order to determine whether the daughter rediae actually ingest liver cells, a few additional infected clams were dissected and living rediae examined with phase-contrast. Liver cells were seen in the blind-sac caeca of several daughter rediae.

Discussion

The destruction of the liver cells is attributed to their being ingested by the daughter rediae since liver cells were seen within the caeca of several living daughter rediae. Apparently the destruction of the clam's hepatopancreas by the parasite was the cause of their death. Is is suspected that the destruction of the bivalves' hepatopancreas and the death of the bivalves are results of repeated infections since such gross destruction of the hepatic mass was not observed by the senior author during his earlier experiences with laboratory-infected clams when the degree of infection was much less and reinfections were not possible. Whether the parasite is actually capable of digesting the host's cells is not known, however, Hsü and Li (1940) suggested that blood, tissue cells, tissue exudates and food particles can be utilized as food by adult intestinal trematodes, and Müller (1923) stated that the liver fluke, *Fasciola hepatica,* feeds primarily on bile duct epithelium, although Stephenson (1947) stated that this liver fluke subsists on blood. Since trematodes apparently have been observed to thrive on whole cells, it is quite possible that the second generation rediae of *C. cornutum* may utilize the liver cells of *Spaerium striatinum* as nutrient.

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Helminth Parasites of Pronghorn Antelope (*Antilocapra americana*) In New Mexico with New Host Records*

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Pronghorn antelope are numerous on the ranges of the Southwest, but little is known of their parasites in this area. In fact, records and reports of the occurrence of helminths in pronghorns from states other than the Dakotas and Wyoming are few.

The literature on helminths of this host was reviewed rather recently by Goldsby and Eveleth (1954), who recovered 15 species of helminths, representing nine genera, from 95 antelope from North Dakota. However, they failed to mention the finding of *Thysanosoma actinioides* in New Mexican antelope by Allen and Kyles (1953). Later, Landram and Honess (1955) recorded the lungworm, *Protostrongylus macrotis,* from antelope in Wyoming, Honess and Winter (1956) listed several additional species of antelope parasites from the same state, and Schad (1958) reported the spinose ear tick, *Ornithodorus* (*Otobius*) *megnini,* from New Mexican pronghorn antelope. The purpose of this paper is to record additional parasites recovered from this host in New Mexico.

MATERIALS AND METHODS

A post-mortem examination was made of 18 antelope killed on four ranches in New Mexico. Most of the animals were killed during the regular hunting seasons in October of 1949 and October of 1953. Two were killed in June of 1954. The ranches on which they were killed were used for sheep or cattle or both.

All of the antelope were examined for internal parasites and nine were examined for external parasites. Examinations were made of 11 sets of lungs and 12 livers with their superficial bile ducts. No examinations were made of the esophagus, rumen, reticulum, and omasum. The remainder of the digestive tract of all animals was searched for helminths. In the earliest examinations, microscopic search was made of the contents of the duodenum and macroscopic search was made of those contents of the abomasum, large intestine, and the rest of the small intestine, which were retained on 20 and 40 mesh screens used in combination. This procedure was later modified by taking fractional samples, varying from 1/10 to 1/20 of the total, of the contents of each organ for small worms and screening the remainder of the material through 10 and 20 mesh screens in combination for recovery of the larger species. The latter method was less time consuming, and probably gave more accurate results for the small species. The number of worms recovered (table 1) are therefore based on direct counts in some instances and on estimates from dilution counts in others.

RESULTS

No external parasites were found. Lungs and livers examined were worm-free except for one larval nematode which could not be identified. All other

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worms encountered were in the abomasum and intestines.

The names of these helminths, the numbers and percentages of the animals that harbored each kind, and the estimated numbers recovered, are listed in table 1. Two cestodes, *Thysanosoma actinioides* and *Moniezia expansa,* and ten species of nematodes, represening seven genera, were recovered. In certain cases, positive specific or even generic identification of female nematodes presents difficulties. Therefore, we tabulated females of *Cooperia* and *Trichostrongylus* separately from males and as specifically unidentified. The combined numbers of *Pseudostertagia-Ostertagia* females are tabulated similarly.

Nematodirella longispiculuata was the most prevalent nematode and *Pseudostertagia bullosa* also occurred in considerable numbers. The species found in lesser numbers were *Cooperia punctata, C. oncophora, C. pectinata, Haemonchus placei* (tentative identification), *Trichostrongylus axei, T. colubriformis, Ostertagia ostertagi* and a species of *Nematodirus* which we have also identified tentatively as *N. lanceolatus* Ault, 1944.

Associated with adult *Nematodirella longispiculata* were large numbers of larvae which were loosely coiled in the preserved state. We have identified them tentatively as late third- and early fourth-stage larvae of this species. However, since parasitic larvae of *Nematodirella* cannot be distinguished with certainty from those of *Nematodirus,* we have assigned these specimens to *Nematodirella-Nematodirus.*

DISCUSSION

Roberts, Turner and McKevett (1954) found evidence that the "bovine strain" of *Haemonchus contortus* is a separate species from the "ovine strain" and proposed the name *Haemonchus placei* (Place, 1893) Ransom, 1911 for the cattle *Haemonchus.* These authors differentiated *H. contortus* from *H. placei* by several characters, including differences in the measurements of the spicules. A study of the spicules of a majority of the males found by us showed their measurements to be in the range specified for *H. placei.* However, since we have not studied the specimens of either sex with respect to any of the other characteristics, it is felt that our identification of them as *H. placei* should be considered tentative.

We have listed *Nematodirella longispiculata* as such in table 1 and elsewhere in this report; however, lengths of the spicules of 45 of the specimens taken at random showed general agreement with the range given by Dikmans (1935) for *N. longispiculata antilocaprae,* a subspecies he proposed for forms from the pronghorn antelope.

We encountered four male specimens of *Nematodirus* whose structure differs from that of the common species of this genus. They resemble the males of *N. oiratianus* Raevskaia, 1929, a species known only in European and Asiatic Russia and also are very similar to *N. lanceolatus* Ault, 1944, a species known only in South America. The lengths of the spicules of our specimens from antelope more nearly approximate the range given by Ault (1944) for *N. lanceolatus* than that given by Raevskaia (1931) for *N. oiratianus.* We are therefore listing our specimens tentatively as the first mentioned species.

Several female *Cooperia* with a linguiform vulvar process characteristic of *C. bisonis* Cram, 1925, were recovered. However, in view of the following comments supplied by Dr. E. W. Price, we are listing these as *C. oncophora:* "Mr. Lucker has studied collections of *C. 'oncophora'* from cattle and of *C. 'bisonis'* from the bison. The results, which cannot be related here in full, suggest that Ransom and Cram dealt with a single variable species. Hence,

Table 1. Helminth parasites recovered from 18 pronghorn antelope in New Mexico

Parasites	No. of Animals infected	Incidence	Worms Recovered: Range	Worms Recovered: Average
Nematodirella longispiculata (M and F)	15	83.3	3-4,740	1,635.8
*Nematodirella-Nematodirus**	16	88.9	2-4,230	443.6
Pseudostertagia-Ostertagia (F)	14	77.8	2-1,200	302.4
Pseudostertagia bullosa (M)	14	77.8	1-660	162.2
Haemonchus placei (M and F)**	14	77.8	8-111	38.2
Cooperia species (F)	16	88.9	1-120	21.9
Trichostrongylus species (F)	7	38.9	1-46	13.6
T. colubriformis (M)	2	11.1	1-18	9.5
Cooperia punctata (M)	13	72.2	1-40	7.9
Trichostrongylus axei (M)	3	16.7	1-10	7.0
C. pectinata (M)	6	33.3	1-20	6.5
C. oncophora (M)	6	33.3	1-10	6.2
Nematodirus lanceolatus (M)**	3	16.7	1-2	1.3
Ostertagia ostertagi (M)	2	11.1	1-1	1.0
Thysanosoma actinioides	2	11.1	1-1	1.0
*Moniezia expansa****	2	11.1	1-1	1.0

M = male; F = female
*Immature.
**Tentative identification.
***One additional immature cestode could not be identified.

he is strongly inclined to agree with Travassos' (1937) action in synonymizing these two names . . . ."

Cooperia pectinata, C. punctata, and *Nematodirus lanceolatus* have not previously been reported from the pronghorn antelope. There is no prior report of the occurrence of *N. lanceolatus* in any host animal in the United States.

Our finding of *Nematodirella* and *Pseudostertagia* in rather large numbers in antelope in New Mexico is in agreement with the findings of Goldsby and Eveleth (1954) in North Dakota. *Nematodirella longispiculata* has likewise been found to occur in relatively large numbers in Montana and South Dakota antelope according to an unpublished list supplied by the late Dr. Lee Seghetti and Honess (1949) found a large number in a Wyoming antelope. Although it has been reported from several other wild ruminants (Dikmans, 1939) and has been found in large numbers in moose (Olsen and Fenstermacher, 1942), it probably is primarily a parasite of antelope. Indications are that it definitely is not primarily a parasite of domestic ruminants. It has not been reported from cattle so far as we are aware. The only record of its occurrence in sheep in this country is that of Honess (1951). He found 17 of 90 (19 per cent) Wyoming sheep infected, which harbored an average of only 35 specimens.

The suggestion that *Pseudostertagia bullosa* is primarily a parasite of the pronghorn was first advanced by Lucker and Dikmans (1945). Goldsby and Eveleth (*loc. cit.*) noted that their results supported this suggestion and our results likewise are in accord with it. Lucker and Dikmans (*loc. cit.*) further indicated that their observations, as well as those of Ransom and Hall (1912), showed a limited distribution and low intensity of *P. bullosa* infection in domestic sheep. They also suggested that this parasite's occurrence in sheep probably is dependent upon its occurrence in antelope. This view seems to be supported by the observations of Olsen (1950) who found relatively small numbers of *P. bullosa* in sheep originating in New Mexico in a region where antelope were established. Also, Honess (1951) found only small

numbers of this worm in sheep in Wyoming. Although Allen (1955) found a 44.4 percent incidence of *P. bullosa* in nine New Mexican mountain sheep (*Ovis canadensis mexicana*), these infections likewise were light (10 to 40 worms) and the possibility that they were acquired from antelope cannot be excluded. This species has not been reported from cattle as far as we know.

The ranges grazed by the antelope examined by us were used predominantly by cattle. It was not surprising therefore to find in these antelope several species of worms that generally are regarded as well adapted to bovine hosts; these include *M. expansa, T. colubriformis, C. punctata, C. pectinata, C. oncophora, O. ostertagi,* and the species tentatively identified as *H. placei.*

Ten of the 18 antelope dealt with in the present report were examined for the fringed tapeworm by Allen and Kyles (1953). Its occurrence in one of these 10 has been reported by them. We found it in one of the remaining 8. Hence, only two of the 18 harbored this cestode, *Thysanosoma actinioides* (table 1). Allen and Kyles (*loc. cit.*) recorded it from only one of the other nine antelope mentioned in their report. Thus, in all, only three of 27 New Mexican antelope examined have yielded this tapeworm; moreover, two harbored only one specimen each. Our present data support the evidence initially provided by Allen and Kyles (*loc. cit.*) that *T. actinioides* is undoubtedly better adapted to domestic sheep than to pronghorn antelope.

Summary

Eighteen pronghorn antelope from New Mexico were examined for parasites. Nine were examined for ectoparasites. All 18 animals harbored helminths and the numbers of mature worms present ranged from one to 4,740. No ectoparasites were found.

Twelve species representing nine genera were recovered. The most prevalent species were *Nematodirella longispiculata* and *Pseudostertagia bullosa.* The others were *Moniezia expansa, Thysanosoma actinioides, Trichostrongylus axei, T. colubriformis, Cooperia punctata, C. pectinata, C. oncophora, Ostertagia ostertagi,* and two species tentatively identified as *Nematodirus lanceolatus* and *Haemonchus placei.*

Cooperia pectinata, C. punctata and *N. lanceolatus* are reported for the first time from the pronghorn antelope. *N. lanceolatus* is reported for the first time in the United States.

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Nemic Galvanotaxis

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The behavior of nemas in an electric field has, to the authors' knowledge, never been determined. Nemic reaction in this respect is reported herein.

MATERIALS AND METHODS

The galvanotactic behavior of *Panagrellus redivivus* (Linn., 1767) Goodey, 1945, was observed on 1 per cent water agar or sandy loam soil in plastic vessels 1.8 cm wide, 15 cm long and 1.6 cm high. The vessels were equipped with a cathode at one end and an anode at the other in such a way that an electric current passed through the substrate.

The source of the direct current was two 6-12 volt rectifiers (battery chargers) and a 6 volt wet cell, also used to stablize current flow. They were used singly or in series as needed. Amperage was controlled by the use of variable and constant resistors. Appropriate ammeters were used to measure current.

P. redivivus were collected on filter paper utilizing a Buchner funnel to remove excess water. Approximately 10,000 nemas were placed at various dispersal points with a probe. These starting points were mid-point of the vessel, 4 cm from the cathode, at the cathode, 4 cm from the anode or at the anode. After 3 hours the agar was divided into thirds and the nemas recovered by agitating with water in a 5-dram vial. Soil, when used, was also divided into thirds. However, the duration of the experiment was 24 hours and the nemas were recovered by use of Baermann funnels. The numbers of nemas were estimated by the dilution method.

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TABLE 1.—Percentage of nemas migrating from the center to the cathode shown under the various milliamperes used. The percentages shown are the averages of three replications. Fiducial limits based on check at one per cent = 54 ± 8.

0	0.01	0.02	0.04	0.08	0.20	0.40	1.00	2.00
54	49	73	82	69	76	86	92	86

Results and Discussion

Agar: A tactic movement to the cathode at 0.02 ma (milliamper) and above (Table 1) resulted when nemas were placed on agar at the mid-point of the container and allowed to migrate under electrical stimuli of various amperages ranging from 0.01 ma to 2.0 ma. Only actively moving nemas were counted in the cathode third and anode third of the agar. Nemas not under the influence of an electric stimulus (controls) distributed themselves at random. Tactic response was limited to a threshold value of 0.02 ma, below which movement was not significantly different from the random movement of the controls.

When 1 ma of current was used and the nemas were placed near the cathode (4 cm), near the anode (4 cm), or at the anode, movement resulted in the following percentages of nemas recovered at the cathode: 95, 84 and 20 per cent, respectively. Migrations of nemas to the cathode from these same positions in vessels containing the controls receiving no electrical current were, respectively, 80, 20 and 2 per cent. When nemas were placed at the cathode, no migration to the anode was observed, the per cent nemas at the cathode being 98 as were 98 per cent in the controls. The migrations to the cathode were statistically significant (at the 1 per cent level) regardless of where the nemas were placed. In no case did significant migration occur toward the anode. No significant differences were found in tactic reactions between males or females.

The same trends occurred when a current of 2 ma was used. Higher amperages resulted in liquefaction of the agar, and at 52 ma, excessive heat killed the nemas.

Nemas of the following genera also showed a tactic movement toward the cathode: *Rhabditus, Neocephalobus, Eucephalobus, Chiloplacus, Tylenchus, Pratylenchus, Aphelenchoides* and *Dorylaimus.*

Soil: Movement was much slower on soil as only 4 per cent of the nemas (average of 5 replications) migrated from the mid-point of the controls in a 24-hour period. However, with 1 ma of current, 29 per cent of the nemas migrated toward and were recovered near the cathode, 71 per cent were recovered near the mid-point and none near the anode. The migration to the cathode was statistically significant.

In view of this phenomenon and the fact that it is present in several genera, it is possible that nemic galvanotactic behavior may be of value in future research with respect to classification, mixed population separation or control.

Summary

The galvanotactic phenomenon of nemas migrating to the cathode in an electric field is reported. This phenomenon was demonstrated on both agar and soil. The threshold of response of *Panagrellus redivivus* was below 0.02 milliamperes. Males and females responded identically to the galvanotactic stimulus.

Notes on the Probable Partial Life-History of *Galactosomum spinetum* (Braun, 1901) (Trematoda) from the West Coast of Florida*

FRANKLIN SOGANDARES-BERNAL† and ROBERT F. HUTTON‡

A single half-beak, *Hyporhampus unifasciatus* (Ransani), (Pisces), from John's Pass, Florida, was found infected with a large heterophyid metacercaria encysted in the visceral adipose tissue. Fifty of these metacercariae were fed to a laboratory raised hamster, and another five dissected from their cysts, observed microscopically and fixed in boiling water. Careful examination of the exposed hamster after 96 hours revealed that no worms were present.

The metacercariae from the half-beak were identified as a species of *Galactosomum* Looss, 1899. Outstanding features of the metacercariae were: (1) excretory vesicle extending to the posterior testis; (2) an immediately pre-equatorial gonotyl position; (3) a very short prepharynx and esophagus, placing cecal bifurcation a long distance from the gonotyl; and (4) the uterus extending anterior to the gonotyl. This latter character was observed in a single live specimen and although difficult to observe seems to be present in our stained material.

Our material from the half-beak was compared with different species of *Galactosomum*, in different stages of development, collected from varied seabirds along the west coast of Florida. The proportions and other details of the metacercariae from the half-beak agreed so closely with specimens of *Galactosomum spinetum* (Braun, 1901) collected from skimmers, *Rynchops nigra* Linn., (Aves), (Figs. 1 to 4: projected scales in mm.), in Gasparilla Sound, Florida, that they are at this time considered the same species. Prudhoe (1949) has indicated that the pregonotylar uterine extent is diagnostic for *G. spinetum* (Braun, 1901). There is a possibility that the metacercariae from the half-beak could represent an undescribed species of *Galactosomum*.

We have found specimens of surface fishes, *Fundulus similis* (Baird and Girard) and clupeid fish remains, in skimmers from Little Gasparilla Pass, Gasparilla Sound, Florida. The presence of *G. spinetum* metacercariae in the half-beak, a predominantly surface fish would form a natural source of infection for the skimmer.

Cable (1956) believed larval forms of *Galactosomum* to be certain magnacercous cercariae. We have collected two of 2731 *Cerithium muscarum* Say from Boca Ciega Bay, Florida, infected with a non-aggregating magnacercous cercaria. A large number of *Fundulus similis* from St. Petersburg, Florida, have been examined for encysted trematodes, and only *Parascocotyle diminuta* (Stunkard and Haviland, 1924) has been found encysted in the gills. For this study, five Fundulus similis were collected from the same locality. Four of these fishes were examined for encysted trematodes and only *P. diminuta* was found in the gills. The remaining *Fundulus similis* was exposed to the non-aggregating cercaria from *Cerithium muscarum* collected in Boca Ciega Bay,

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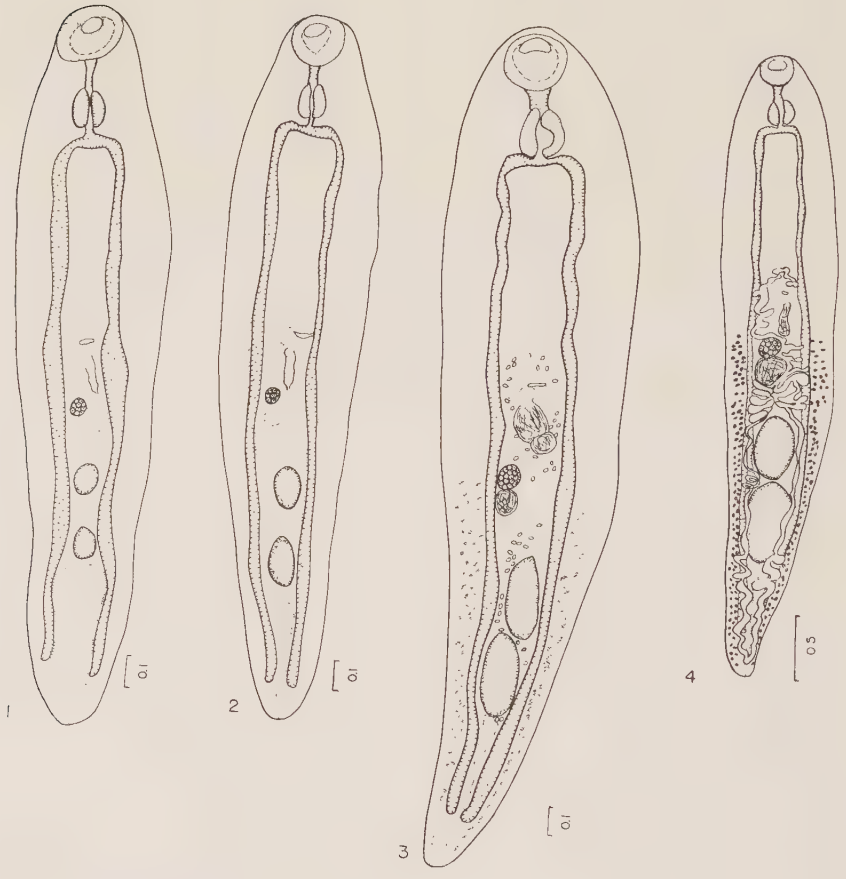
Florida. Examination of this fish host after about 12 days exposure to the magnacercous cercaria revealed the presence of ten metacercariae excysted in the musculature adjacent to the interhaemal spines of the anal fin. One of these live worms was dissected from the cyst and examined microscopically and the others fed to a chick (roughly three days old). The metacercaria from *Fundulus similis* and *Hyporhampus unifasciatus* could not be confused. The one from *Fundulus* may represent another species of *Galactosomum* which is commonly found in gulls of this area. Examination of the exposed chick after three days revealed that no worms were present in the digestive tract. Although the metacercaria from the half-beak did not prove to be identical



All drawings made with the aid of a camera lucida. The projected scale has value in millimeters.

Fig. 1. Metacercaria from half-beak tentatively identified as *G. spinetum*. Ventral view.

Fig. 2. *G. spinetum* from skimmer. Immature specimen. Ventral view.

Fig. 3. Same. Mature specimen with a few eggs in the uterus and poorly differentiated vitellaria. Ventral view.

Fig. 4. Same. Fully developed specimen. Ventral view.

with the one experimentally obtained in *Fundulus similis,* by exposure to a non-aggregating magnacercous cercaria, it would not be inconceivable to believe that half-beaks could possibly infect themselves by feeding on positively phototrophic magnacercous cercariae.

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The Genus *Perodira* Baylis, 1943 (Nematoda: Drilonematidae), with a Description of a New Species

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Baylis (1943) described a new genus of drilonematid nematode parasites from the body cavity of the South Indian earthworms, *Hoplochaetella anomola* and *Drawida* sp. The type and only species, *Perodira alata,* had such typical drilonematid features as caudal pits or suckers ("pocket-like" phasmids of Chitwood & Chitwood, 1950), a single ovary, and a long narrow esophagus. Yet it differed markedly from other drilonematid genera in the possession of a U-shaped stoma, and especially in the presence of large pouch-like amphids. Amphids of this shape are characteristically found in many marine nematodes of the Class Aphasmidea, Superfamily Enoploidea. Among the Phasmidea these amphids are most similar to the transversely-elliptical amphids of some diplogasterids.

In dissecting the earthworm *Pheretima posthuma* (L. Vaillant), obtained from Subida, Kamalapur, and Golla, all in Dacca District, we found large numbers of a nematode which strongly resembled *Perodira alata* in even the finest details. The general body form, the strong coiling in the male, and the shape of male and female tails were the same. In both, the amphids were large and pouch-like; the excretory pore was located behind the esophageal base; the ovary extended well into the tail and was not reflexed; the phasmids were "pocket-like" and asymmetrically placed on the two sides of the tail; there was a prominent ejaculatory duct in the male but no copulatory apparatus; and alae were found on the male tail. However, Baylis could not locate an anus, whereas in our specimens the anus in the female was quite prominent. The head was not set off by a constriction in *P. alata* and there was a small U-shaped stoma, unlike our species which had no stoma. The esophagus had a narrow isthmus in *P. alata,* but in our species it was clavate. Baylis likewise did not report subventral excretory cells, which in our species were large and definite. Moreover, Baylis' specimens were stated to have been free in the body cavity, whereas ours were located in the testis sacs and seminal vesicles, especially in the latter. Only one other genus, *Dicelis,* has been found in a similar habitat. *D. filaria* Dujardin, 1845, was reported from the testis sac of *Lumbricus* (Wülker, 1926), while *D. nira* Chitwood & Lucker, 1934, was reported from the gonads of *Helodrilus* (probably the seminal vesicles, since the testes degenerate in mature earthworms, the ovaries are quite small, and testis sacs are not present in *Helodrilus*).

The body of *Perodira* is extremely delicate* and some specimens in our collection which underwent distortion after formalin fixation showed a V-shaped stoma and a bluntly-rounded male tail, much as in Baylis' drawings. Because of the obvious resemblances mentioned above, we have identified our specimens with *Perodira* and offer the following emendation of the original genus diagnosis.

The Genus *Perodira* emended

Diagnosis: Drilonematidae. Mouth terminal; stoma lacking. Esophagus clavate. Amphids pouch-like; phasmids "pocket-like" and usually asymmetrically placed. Tail of male blunt, with conoid process at tip. Caudal alae in male, supported by setose genital papillae. Copulatory apparatus absent. Vulva equatorial. Female genital tube single, prodelphic, reflexed anteriorly.

Perodira pheretimae, n. sp.

Description. Body transparent and delicate; cuticle with very fine transverse striation and more prominent longitudinal subcuticular striation. Head set off by constriction; bearing two circles of papillae, an inner circle of six tiny circumorals and an outer circle of probably four papillae. Paired lateral amphids large and slit-like, with concave aperture situated at level of cephalic constriction; a broad amphidial pouch containing a sensilla leads posteriorly from each amphid. Stoma lacking, but with slight central depression at anterior end of esophagus. Esophagus expanded slightly in head region, and then of uniform width until it broadens out at base. One posterior dorsal and two anterior subventral gland nuclei present in expanded base. Esophagus surrounded by many small indistinct cells, and overlapped at base by excretory cells. Nerve ring surrounds esophagus just anterior to expanded base. Excretory pore located about 30 microns posterior to esophageal base in both sexes. A broad subventral excretory cell on either side runs anteriad and posteriad from excretory pore. Large paired excretory cell nuclei located just posterior to pore with excretory canals running through excretory cells. Intestine narrow and indistinct, with a wavy refringent lining, cells in life contain scattered pale green globules. Esophagointestinal valve small, and together with the esophageal base, displaced somewhat dorsally by excretory cells. Intestine runs dorsally above testis in male, and in female runs dorsally above ovary and ventrally to anus behind vulva. There appear to be four intestinal cells in cross-section.

Female reproductive system simple; single ovary begins in middle of tail and contains a single row of oocytes; oviduct runs forward to near excretory pore. There appears to be a small constriction between vagina and uterus, which serves as a seminal receptacle; no seminal receptacle at anterior loop of oviduct, as reported for *P. alata.* Uterus muscular and contains only one fully-formed ovum at a time; vulva and vagina inclined anteriorly. Tail long and narrow, and tapers to a fine obtuse tip. Posterior wall of anus protrudes as prominent anal lip. Phasmids asymmetrically placed, one on right side being more posterior than that on left.

Male reproductive system consists of single testis, anteriorly reflexed to right side for short distance; three or four rows of spermatocytes appear in lateral aspect for most of length of testis; muscular ejaculatory duct found

*In death the worms are loosely-coiled and in life they undergo violent contortions and quickly burst when dissected out in tap water.

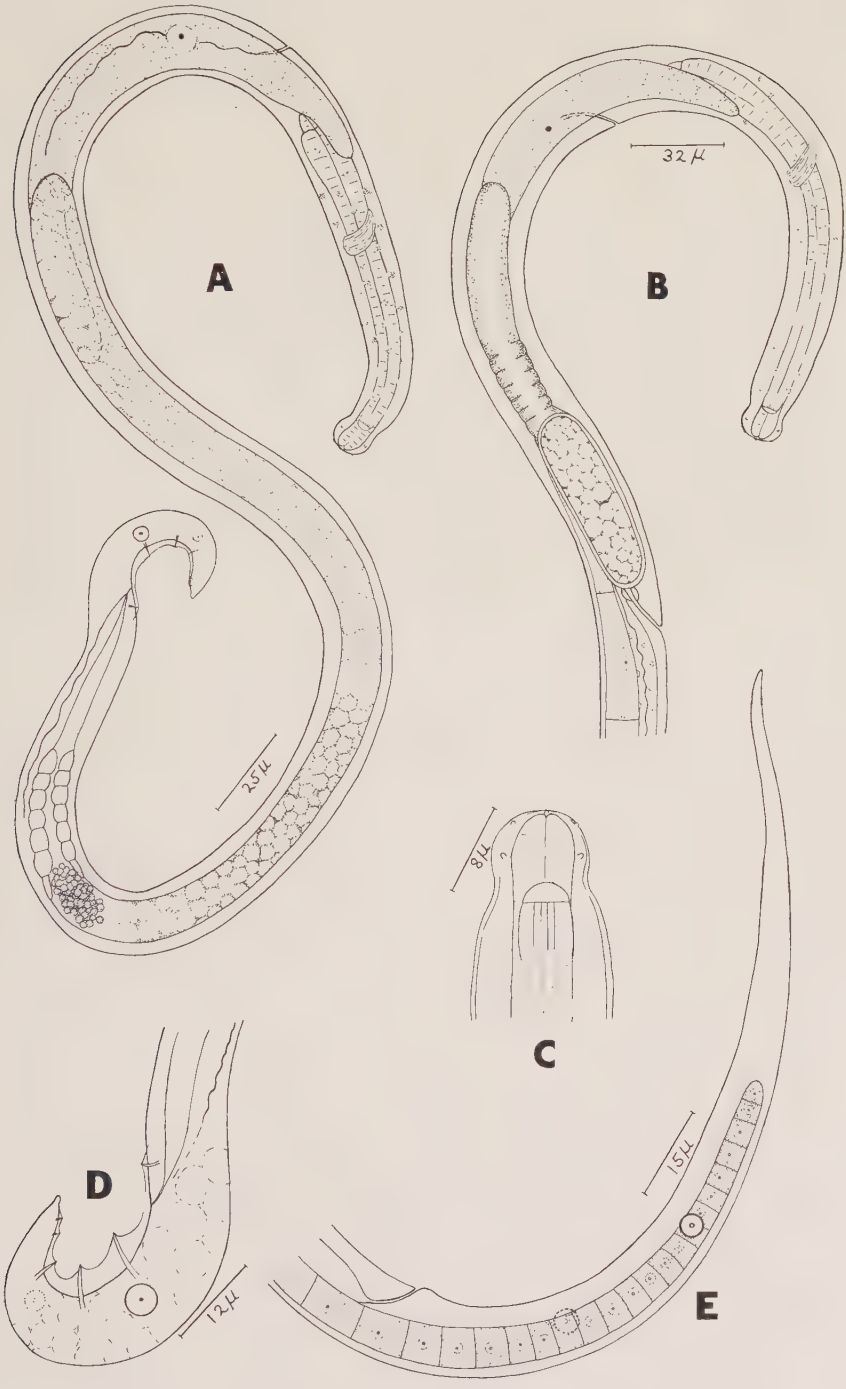

Fig. 1. *Perodira pheretimae* n.sp. A. Entire male. B. Anterior half of female. C. Head of female. D. Male tail. E. Female tail.

short distance anterior to anus, and narrow vas deferens leads from it to cloaca. Sperm, as seen in male just anterior to ejaculatory duct or in uterus of female, spherical or amoeboid. Cloacal junction of intestine and vas deferens indistinct. Spicules and gubernaculum lacking. A pair of delicate caudal alae present, supported by six pairs of fine setose genital papillae, one pair preanal and five pairs postanal, last two very tiny; forward extension of alae could not be traced. (In addition to the setose caudal papillae there are frequently seen in both male and female somatic "setae" of various lengths and distribution, but these represent a kind of filiform fungus attached to the nematode.) As in female, phasmids asymmetrically placed, with right one being more posterior. Tail ends in small conoid process.

FEMALE DIMENSIONS: Length 714-795 microns. Maximum body diameter 19-26 microns. Anal diameter 13 microns. Body diameter at esophageal base 19 microns. Esophagus 80-128 microns. Tail 135-152 microns. Vulva 48-49%. Ovum with clear shell 61 x 19 microns.

MALE DIMENSIONS: Length 610-668 microns. Maximum body diameter 16-19 microns. Esophagus 88-96 microns. Tail 60-76 microns. Total length of testis 58-62% of body length. Testis reflex 50-60 microns.

HOLOTYPE FEMALE: Length 714 microns. Maximum body diameter 22 microns. Esophagus 80 microns. Tail 135 microns. Vulva 48%. One ovum with shell in uterus.

ALLOTYPE MALE: Length 666 microns. Maximum body diameter 19 microns. Esophagus 96 microns. Tail 60 microns.

TYPE SPECIMENS: Holotype female and allotype male: personal collection, A-30; Paratypes: (male and female): A-31, 32, 33.

TYPE HOST: *Pheretima posthuma* (L. Vaillant).

TYPE LOCALITY: Subida, Dacca, East Pakistan.

TYPE HABITAT: Testis sacs and seminal vesicles.

DIAGNOSIS: The present species differs from *Perodira alata* in the relatively narrower body and smaller size of both male and female, in the more anterior position of the vulva, in the lesser number of fully-formed ova in the uterus, and in the greater proportion of length to breadth of the ova (more than 3:1 in *P. pheretimae,* as opposed to 2:1 or less in *P. alata*).

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Survival of Encysted and Free Larvae of the Golden Nematode in relation to Temperature and Relative Humidity*

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The golden nematode (*Heterodera rostochiensis* Wr.), in completing its life cycle, spends approximately 320 days in the soil subjected to the fluctuating temperature and moisture existing there. The remaining 45 days are spent

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in direct association with roots. While in the soil, the larvae of the golden nematode are enclosed in a protective cyst, which is the transformed body wall of the dead swollen female. The first molt of the young larva occurs inside the egg, usually when the swollen female is yellow in color (Hagemeyer 1951). The second-stage larva is found within the brown cyst, either coiled inside the egg shell or free in the cyst itself. The studies reported here have been performed on encysted second-stage larvae, except one experiment in which unprotected second-stage larvae were used.

The purpose of these studies was to obtain additional information on the effect of temperature and relative humidity upon the retention of viability of encysted larvae. The majority of the experiments were conducted in the laboratory, where these factors can be separately controlled and maintained. The results of preliminary experiments have been reported (Mai, 1952; Mai and von Mechow, 1952).

MATERIALS AND METHODS

The large numbers of encysted larvae needed in these experiments were separated from infested soil by a flotation method described by Lownsbery (1950). When necessary, small quantities of cysts were separated from soil by washing the soil through a combination of U. S. No. 25 and No. 60 sieves. Except where noted, cysts were washed from infested field soil; thus cysts of different ages were included. Relative humidities were maintained by different concentrations of sulfuric acid in large moist chambers (Stevens, 1916). Temperature was controlled by placing the moist chambers in constant-temperature cabinets, without light.

Three criteria, larval emigration, microscopic examination of cyst contents, and root infection, were used to determine larval viability. In the larval-emigration method, water percolated through soil in which potato roots were growing actively was used to stimulate the emergence of larvae from cysts (Lownsbery 1951). Fresh leachings were used in experiments conducted during 1950-1951, and frozen leachings were used in subsequent experiments conducted from 1952 through 1955. Like quantities of cysts, either a counted number or a weighed amount of cyst material, were placed in leachings for three weeks. In larval-emigration tests conducted from 1950 through 1952, the cysts were placed in 25 ml. of leachings added at the beginning of the three-week period. In tests conducted after 1952, the cysts were placed in 10 ml. of leachings to which an additional 10 ml. was added at the end of the first week and again at the end of the second week, marking a total application of 30 ml. The cysts were kept at a constant temperature of 75°F. All larvae were counted in dishes containing less than 100 larvae; when numbers were higher than this, counts were made of the larvae in suitable aliquots.

The microscopic examination of cyst contents involved opening each cyst with a dissecting needle or fine scalpel and examining the contents under a stereoscopic microscope. Cysts were soaked in water for 16 hours before they were opened. A larva was classified as dead when no movement was seen and when one or more of the following observations were made: (1) the end portion of the tail was opaque, (2) the inner wall was pulled away from the outer wall, (3) the stylet was beginning to disintegrate, (4) there was no flow of body contents when the larva was cut transversely with a sharp scalpel.

In the root-infection method, known quantities of the treated cysts were added to pots of sterilized soil into which a potato seed piece *Solanum tuber-*

osum (L. (var. Katahdin) was planted or into which a young potato plant was transplanted (Mai, 1951). Because of the more uniform growth of the transplants, measurements of larval viability were more uniform when such plants were used than when seed pieces were placed directly into the pot.

Results

Free and encysted larvae removed from soil: Both encysted and free golden nematode larvae were maintained at relative humidities of 100, 88, 20, 3, and 0-1.5 per cent at temperatures of 75°F. and 40°F. in the first experiment which was conducted in 1950-1951. Fifteen lots of 1500 free larvae were exposed to the various treatments on filter paper in moisture chambers. After exposure to the treatments for 233 days the viability of the larvae was tested by the root infection method. No females were found on potato roots growing in soil to which larvae from any of the treatments were added. An average of 21 females developed on each potato root system growing in soil to which freshly-emigrated larvae were added.

In the same experiment encysted larvae were exposed for as long as 324 days to the same environmental conditions as the free larvae. After 16, 75, 116, 194, 240, and 324 days, viability was determined by the larval-emigration method. After 324 days viability was tested also by microscopic examination and by the root-infection method. As indicated by larval emigration the viability of encysted larvae at all treatments was essentially the same after 16, 75, and 116 days. After a period of 194 days, emergence was about the same for all treatments except relative humidities of 88% and 0-1.5% at 75°; under these conditions, fewer larvae emerged than under the other conditions. This pattern of larval emergence was still more evident after 324 days (Expt. 1, Table 1), with no larvae emerging from cysts stored at 75° when the relative humidity was 88% or 0-1.5%. In addition, an effect of humidity at 40° became evident; emigration at 0-1.5% relative humidity was negligible at

Table 1. Rate of Larval emergence from samples of 600 cysts in experiment 1, 150 cysts in experiment 2, and 2100 cysts in experiment 3.

Relative Humidity (%)	Emerged larvae per 100 cysts			
	Expt. 1 After 324 days at indicated temp.		Expt. 2 After 233 days	Expt. 3 After 177 days
	40°F.	75°F.	at 75°F.	at 75°F.
100	640	571	790	318
96.2*			288	616
94.8				672
92.3				41
88*	813	0	1	27
82.3				410
80.5				332
78.7*			16	637
45*			724	
20*	768	717	907	659
7.5*			1120	
3	425	128	1002	
0-1.5*	1	0	0.5	0

*These percentage relative humidities were reported as 98.2, 94.8, 91.2, 74.6, 49, 25, 10.5, and 1.5, respectively, by Mai and von Mechow (1952). The concentrations of sulphuric acid and distilled water, erroneously made up on a volumetric basis, have been recalculated and placed on a weight basis.

either temperature. The decline in larval viability at 88% relative humidity at 75° and at 0-1.5% relative humidity at both temperatures was also demonstrated by microscopic examination of cyst contents and by root-infection tests at 324 days.

A second experiment involving the exposure of encysted larvae to various relative humidities at 75°F. was conducted in 1951-1952 to verify results obtained in the previous experiment. As in the first experiment, the encysted larvae died more quickly at relative humidities of 88 and 0-1.5% than at any other tested (Expt. 2, Table 1). At 88% relative humidity the decrease in number of emigrated larvae was noticeable in 88 days and became progressively more pronounced after 128, 159, 186, and 233 days. At 0-1.5% relative humidity a decline in the numbers of larvae was evident in 159 days and continued to be apparent in the later tests. A decrease in the number of emerged larvae became evident after the encysted larvea had been exposed at 78.7% relative humidity for 186 days. Except for a moderate decrease in emigration at 96.2%, larvae at the other relative humidities emerged from the cysts at a high rate. The results of the microscopic examination of cyst contents and the root-infection tests of cysts exposed for 233 days at each treatment were in accord with the numbers of emerged larvae.

A third experiment was conducted in 1952-1953 to verify results obtained in the first two experiments. The procedure followed in this experiment was similar to that followed in the two previous ones. At the termination of the treatments after 177 days, viability was determined by the three previously described methods.

There were no marked differences among the different treatments in the number of larvae that emerged from the cysts at the end of 100 days. By the end of 177 days, however, lower numbers of larvae emerged from the cysts at relative humidities of 88, 92.3, and 0-1.5% at 75° than from cysts receiving the other treatments (Expt. 3, Table 1). In contrast to results obtained in the previous experiment, high numbers of larvae emerged from cysts stored at 96.2% relative humidity at 75°F. It is possible that a reduction in viability at this relative humidity would have been evident had this experiment been carried out for a longer period of time. High numbers of larvae emerged from all cysts stored at 40°F. for 177 days. Viability determinations by the other two methods were in general agreement with the above.

Results from previous experiments at 75° and 40° indicated the need for more information concerning the temperature at which the reduction of viability of encysted larvae stored at relative humidities of 88 and 0-1.5% is most rapid. Also, a need for information on rate of viability decline as 20% relative humidity at temperatures higher than 75° was indicated. Thus, tests were made at 86° and at two temperatures between 40° and 75°.

Of three similar experiments in 1953-1955, the first was for a period of 119 days, the second for 122 days, and the third for 150 days. Viability was determined by all three methods in the first test and only by the larval-emigration method in the last two. In the first experiment 200 cysts were sampled, in the second, 3500 cysts, and in the third, 25 one year old cysts.

In all three experiments, the viability of encysted larvae stored at 86° at relative humidities of 88 and 0-1.5% declined rapidly. The average emergence rate is shown in table 2. Few or no larvae emerged from the cysts subjected to these humidities while large numbers emerged at a relative humidity of 20%. Similar differences were evident at 59°, but they were not nearly as great as at 86°; at 54°, relative humidity had little or no effect on viability.

Table 2. Rate of larval emergence after 4 to 5 months.

Humidity Relative (%)	Emerged larvae per 100 cysts		
	86°F.	59°F.	54°F.
88	1	338	1702
20	1903	1449	2735
0-1.5	3	256	1706

Trends in viability demonstrated by the numbers of emerged larvae were also shown by the number of cysts containing viable larvae and by the numbers of immature females on potato roots.

SURVIVAL OF ENCYSTED LARVAE IN DRY, MOIST, AND FLOODED SOIL: Mai (1952) found that in moist soil viability of encysted larvae decreased as temperature was increased from 37 to 99°F. Additional tests were made on the survival of larvae in soil maintained at various moisture levels.

Fifteen grams of cyst material, containing approximately 67,500 cysts, was added to each of 15 one-gallon crocks of soil. Five of the crocks were filled with air-dried soil to which no water was added throughout the experiment. The soil in five other crocks was maintained at a moisture level satisfactory for the growth of potato plants. The soil in the remaining crocks was completely under water during the entire experiment.

The crocks were placed on a greenhouse bench in October, 1953, and remained there until June, 1954, a total of 245 days. Cyst material then was washed from the soil from each crock and placed in leachings. Viability was determined also by the root-infection method. The soil remaining in each crock was divided into four equal parts and each part was placed in a four-inch pot, making a total of 20 pots per treatment.

Results by both methods indicated that the viability of encysted larvae decreased more rapidly in the moist and flooded soil than in the dry soil. Data obtained by exposing the cysts to leachings are given in Table 3. An additional experiment gave similar results.

DISCUSSION

From the decline in viability of the encysted larvae subjected to some of the treatments, one could conclude that the combination of temperature and relative humidity over a relatively long period of time plays an important role in influencing viability. Relative humidity in the soil is usually high, thus death of encysted golden nematode larvae may result when soil temperature is high for an extended period. These factors, together with the narrow host range of the pathogen, may be important in determining the areas in the world in which this nematode will become a major pest.

No satisfactory explanation can be given for the more rapid death of larvae exposed to 88% (and 92.3% in one test) than at lower or higher relative humidities. As far as we are aware, there is no biological phenomenon associated with relative humidities in this range that would cause this more rapid death. Similar data were obtained in the six separate experiments reported in this paper as well as in several additional unreported nonreplicated tests. In addition, similar data were obtained when relative humidities were maintained by salt solutions.

Examination under high magnification shows that larvae that have been subjected to relative humidities of 88 or 0-1.5% at 75°F. possess highly vacuolated intestines containing little food. At a relative humidity of ap-

Table 3. Rate of larval emergence from 3500 cysts after 245 days in dry, moist, and flooded soil.

	Emerged larvae per 100 cysts	Immature females per root ball
Dry soil	1198	436
Moist soil	407	125
Flooded soil	241	39

proximately 88% at high temperatures, conditions may be favorable for a high metabolic rate and death may be due to starvation. These same conditions also may be favorable for the growth of a fungus or other organism detrimental to golden nematode larvae; however, the presence of such organisms was not noted. At 0-1.5% relative humidity at higher temperatures, death may be caused by desiccation.

Encysted larvae may die rapidly in the moist and flooded soil because of the more favorable environment for the growth of fungi and other organisms detrimental to the golden nematode. Undoubtedly large numbers of larvae emerged from cysts in the moist and wet soils; such nematodes free in the soil would be vulnerable to attack by many kinds of soil organisms. Only the viability of encysted larvae was measured at the end of the experiment. In the flooded soil, some death may have resulted from lack of oxygen.

SUMMARY

When separated from soil and stored at a temperature of 75° or 86°F., encysted larvae of the golden nematode declined in viability much more rapidly at relative humidities of 88% or 0-1.5% than they did at the other relative humidities tested. The decrease in viability was not apparent until the larvae had been subjected to these conditions for several weeks. Viability decreased more rapidly at 86° and 75° than at 59° or 54°F. Except that viability decreased appreciably during storage at 40°F. and relative humidity of 0-1.5% for 324 days, retention of viability was not otherwise affected by relative humidity at this temperature.

Unprotected larvae did not survive exposure for 243 days to relative humidities of 100, 88, 20, and 3% at either 75° or 40°F.

Viability of encysted larvae declined more rapidly in flooded and moist soil than in air-dried soil. Some encysted larvae were viable after being under water for eight months.

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Ascaridia galli (Schrank, 1788) Freeborn, 1923 Inside the Egg of the Chicken

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This *Ascaridia galli* is a white male, 56 mm. long. It was motionless, possibly dead on discovery, but well preserved when given to the author. The pale brown egg, presumably from a Rhode Island Red pullet had been collected and delivered the morning it was opened. The egg was graded between "medium" and "large," probably weighing slightly less than 2 ounces.

Round worms in hens eggs have been reported several times in the past, *viz* Hall (1925), Schell (1951), Penner (1953), Wood and Mizelle (1955), Hall (1945), and Bovee (1954). In one case described personally by Dr. A. Dardiri of this University, the worm is said to have been located between the shell membrane and the shell, faintly molding its shape into the shell, appearing in low bas relief on the outside, and in two other known instances it has been recovered from the buccal cavity of the person eating the egg.

Presumably this intestinal parasite migrated from the cloaca into the uterus, then up the oviduct, meeting the post-ovulated ovum close to the oviducal glands near the infundibulum. It seems most likely that the worm's progress up the tube was rapid because after an egg is received, the oviduct becomes inactive, not resuming its more violent peristaltic contractions until about the time of laying. A further possibility is that the worm did not get past the isthmus at the distal end of the oviduct but was included in the egg white. The demonstration in a few cases of double-yolked eggs that the oviduct can pick up ova escaped into the body cavity indicates a possible path for entrance of the parasite. Because *Ascaridia galli* rarely occurs in the body cavity this access is unlikely.

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Some Hemiurid Trematodes from Hawaiian Fishes*

H. W. MANTER and MARY HANSON PRITCHARD

This paper describes hemiurid trematodes collected by the junior author in Hawaii in the summer of 1949 at the Marine Laboratory of the University of Hawaii. The hosts were identified by Dr. William A. Gosline. All except two of the seven species are described as new. Two of them also occur along the tropical American Pacific Coast, one also in the Gulf of Mexico.

All measurements are in mms. unless otherwise stated.

THE SUPERFAMILY HEMIUROIDEA

The family Hemiuridae (in the sense of Looss and Lühe) is a large group of Digenea almost wholly parasites of marine fishes. Several subfamilies have long been recognized. Recently, the trend has been to elevate these to families of a larger group, the Hemiuroidea or Hemiurata. Skrjabin and Guschanskaja (1954) included 17 families in the suborder Hemiurata (Markevitsch, 1951) Skr. & Gusch., 1954. In 1956, these same authors named the superfamily Azygioidea, placing in it the Azygiidae, Xenoperidae, Hirudinellidae, and Liocercidae. A new subfamily (of Liocercidae), Arnoldiinae, was named for the genus *Arnoldia* Vlasenko, 1931. Such a classification minimizes (incorrectly, we believe) the significance of the vitellaria. *Xenopera* has been generally considered to be a fellodistomid, and Yamaguti (1958) lists it as a synonym of *Proctoeces* Looss, 1901. Hirudinellidae have tubular vitellaria and seem better grouped in the Hemiuroidea. LaRue (1957) named a suborder Azygiata but included in it only the Azygiidae and Bivesiculidae.

We believe the family "Liocercidae" is unjustified; in any case, the name is incorrect. As noted by Ejsmont (1931) *Liopyge* Looss, 1899 is not invalidated by *Liopygus* Lewis, 1891 and its second name, *Liocerca* Looss, 1902, is a synonym of *Liopyge*. Yamaguti (1953) points out that the "cirrus sac" of Looss is actually a sinus sac (or hermaphroditic sac) as in other hemiurids. Although Ejsmont (1931) named the subfamily Liopyginae for this genus, we would classify it in the Lecithasterinae. A prestesticular ovary is known to occur in other genera of Lecithasterinae, e.g. *Gonocerca*.

The subfamily "Arnoldiinae" was based on the preoccupied name *Arnoldia* Vlasenko, 1931, which was renamed *Arnola* by Strand (1942). Yamaguti (1958) named for it the subfamily Arnolinae. Both the Russian authors and Yamaguti placed great importance on Vlasenko's description of a true cirrus sac in this genus; other characters are similar to the Lecithasterinae. Although Vlasenko's figure seems to show a cirrus sac, the terminal part of the uterus is not shown. This species should be restudied to make certain that the sac does not enclose an hermaphroditic duct. The genus is very distinct from Azygiidae, for example in the character of the vitellaria.

Chauhan's (1954) monograph on the Hemiuridae recognizes eight subfamilies and includes a history of the group.

LaRue (1957) accepts the suborder Hemiurata and includes in it nine families. He names a suborder Azygiata for the Azygiidae and the Bivesiculidae. His classification does not pretend to be complete and several families of Hemiurata (as Accacoeliidae, Syncoeliidae, Hirudinellidae) are not mentioned. He adds the family Didymozoidae to the Hemiurata.

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Yamaguti (1958) does not attempt superfamily groupings but recognizes 19 subfamilies of Hemiuridae, of which 9 are new, 6 with a single genus.

Of these four major recent attempts to classify this group of trematodes, LaRue's is incomplete, while Skrjabin and Guschanskaja's and Yamaguti's differ in that the Russians recognize numerous families while Yamaguti prefers numerous subfamilies. Such numerous suprageneric groups, many with a single genus, do not seem justified to us at the present time.

The criteria used in this group to establish families, subfamilies, and genera have been, and probably must be, arbitrary. Most of them show intergradations. It is unsafe to emphasize a "cirrus sac" in this group because several times a structure so-called has proved to be a sinus sac (= hermaphroditic pouch = bursa hermaphroditicus) which the uterus enters to join the male duct. Any report of a hemiurid with a cirrus sac should be supported by serial sections. We believe important characters are (1) cuticular plications (as in *Hemiurus*) as compared with a smooth skin (as in *Derogenes*); (2) an ecsoma, or not; (3) vitellaria compact, lobed, digitate, or tubular. Although Yamaguti minimizes external features such as the cuticular plications and a preacetabular pit, these structures are rather easily seen and probably as dependable as most internal structures. Slusarski (1957) was troubled by what he considered to be a rudimentary ecsoma in *Aphanurus balticus*. The modified posterior portion of the excretory vesicle in this species does not appear to have an outer wall continuous with the cuticula of the body. Even a small ecsoma should be a retracted portion of the body.

The following taxa, similar to those of Chauhan (1954), are suggested:
Superfamily Hemiuroidea

Families: Hemiuridae Lühe, 1901; Accacoeliidae Dollfus, 1923; Aerobiotrematidae Yam., 1958; Didymozoidae Poche, 1907; Hirudinellidae Dollfus, 1932; Isoparorchidae Poche, 1925; Prosogonotrematidae Vigueras, 1940; Syncoeliidae Dollfus, 1923. (We prefer to assign the Ptychogonimidae Dollfus, 1936 to the Azygiata.)

Subfamilies of Hemiuridae: Hemiurinae Looss, 1907; Aphanurinae Skr. & Gusch., 1954, (syn. Ahemiurinae Chauhan, 1954); Dinurinae Looss, 1907; Lecithasterinae Odhner, 1905 (syn. Derogenetinae Odhner, 1921); Lecithochiriinae Lühe, 1901; Prosorchiinae Yam., 1934.

SUBFAMILY HEMIURINAE Looss, 1907. This subfamily is characterized by cuticular plications, ecsoma, and compact or lobed vitellaria. Genera: *Hemiurus* Rud., 1809; *Brachyphallus* Odhner, 1905; *Elytrophalloides* Szidat, 1955; *Glomericirrus* Yam., 1937; *Parahemiurus* Vaz & Pereira, 1930.

The genus *Brachyphallus* has been considered to lack a sinus sac, but Slusarski (1958) shows that the type species, *B. crenatus,* has both a sinus sac (= hermaphroditic sac) and a prostatic vesicle. A study of specimens of this same species collected at Friday Harbor, Washington, confirms this observation. *Brachyphallus* accordingly differs from *Hemiurus* chiefly in possessing a preacetabular pit.

SUBFAMILY LECITHOCHIRIINAE LÜHE, 1901 (syn. Sterrhurinae Looss, 1907). This subfamily is characterized by nonplicated cuticula; ecsoma; vitellaria compact, lobed or digitate. Genera: *Lecithochirium* Lühe, 1901; *Adinosoma* Manter, 1947; *Allostomachicola* Yam., 1958; *Ceratotrema* Jones, 1933; *Dinosoma* Manter, 1934; *Dissosaccus* Manter, 1947; *Elytrophallus* Manter, 1940; *Johniophyllum* Skr. & Gusch., 1954; *Lethadena* Manter, 1947; *Musculovesicula*

Yam., 1940; *Plerurus* Looss, 1907; *Plicatrium* n. gen. (for *Lecithochirium lycodontis* Myers and Wolfgang, 1953); *Separogermiductus* Skr. & Gusch., 1955; *Sterrhurus* Looss, 1907; *Stomachicola* Yam., 1934; *Synaptobothrium* v. Linstow, 1904; *Tricotyledonia* Fyfe, 1954 (syn. *Grassitrema* Yeh, 1955).*

Lecithochirium lycodontis (Figs. 1-3) from a moray eel in New Hebrides might appear from its figure to possess cuticular plications. A loan of type and paratype specimens permitted a restudy of this trematode. The type specimen is not the one figured in the original description. The spine-like structures are rounded papillae irregularly scattered but most common on the dorsal surface of the forebody and the ventral surface of the posterior half of the body. They were visible on 3 of 8 specimens. The vitellaria, viewed from a favorable angle, are typical, seven digitate lobes (Fig. 3) as in *Lecithochirium*. Our measurements of eggs of *P. lycodontis* are 15 to 19 by 8 to 11 microns. Because of pecularities of the terminal genital ducts, a new genus, *Plicatrium*, is proposed for this species.

Generic Diagnosis of Plicatrium: Non-plicated cuticula; ecsoma present; preacetabular pit absent; cuticular papillae sometimes present; testes near acetabulum, symmetrical or diagonal; vitellaria postovarian, consisting of seven digitate lobes narrowed proximally and joined at bases; seminal vesicle bipartite, dorsal to acetabulum, anterior part thick-walled; sinus sac globular, thick-walled, containing prostatic vesicle, prostatic cells, and terminal part of metraterm which joins the male system just anterior to prostatic vesicle; a short duct leads to a much folded, voluminous, eversible atrium; metraterm outside sinus sac surrounded by gland cells (Fig. 1).

This genus differs from *Lecithochirium* in its spacious, eversible genital atrium, prostatic cells inside sinus sac, and lack of preacetabular pit.

THE GENERA *Sterrhurus, Lecithochirium,* AND RELATED GENERA

Sterrhurus and *Lecithochirium* are smooth bodied, tailed hemiurids, with a sinus sac more or less developed, with lobed to digitate vitellaria. Although Skrjabin and Guschanskaja (1954, 1955) assumed a sinus sac to be lacking in *Sterrhurus musculus*, type of the genus, Looss (1907) did in fact describe and figure muscles forming a definite sac-like structure. He wrote, "the muscle fibers in their totality mark off . . . a cirrus-sac-like structure from the surrounding parenchyma . . . Posteriorly the majority of them bend inward toward the entrance point of the pars prostatica."

Slusarski (1958) has shown that *Brachyphallus* also has a sinus sac, but the two genera are easily distinguished by the conspicuous plications of *Brachyphallus* which is actually more closely related to *Hemiurus*.

Both *Sterrhurus* and *Lecithochirium* now contain many species among which the degree of development of the sinus sac varies greatly. In most species it is complete and distinct, but in others it consists of strands of muscles which may not meet posteriorly (the "open type") and in some cases the muscles do not come together to form a sac but consist of only strands in the parenchyma, as in *Synaptobothrium* and *Plerurus*. A sac-like structure is entirely lacking in *Dinosoma, Adinosoma, Lethadena,* and *Johniophyllum*.

Looss (1907) described the morphology of *Sterrhurus* in considerable detail. The character of the terminal genital ducts are of particular interest. Variations in the sinus sac among genera of hemiurids have been mentioned above.

*Yeh added a personal footnote to the reprints he sent out: "*Grassitrema* Yeh. 1955 (Feb./Mar.) is syn. of *Tricotyledonia* Fyfe, 1954." Records of receipt at the Library of Congress indicate some such date.

Typically, the seminal vesicle is a swollen tube partially divided by constrictions or at least by bends into a large posterior part and two decreasingly smaller parts; the narrowed anterior end leads to a pars prostatica surrounded by prostatic cells just outside the sinus sac. The small, slightly swollen, ovoid pars prostatica is filled with what appear to be thin-walled, transparent cells. It enters the sinus sac in the base of which it enlarges to form a spherical vesicle. The lining of this "prostatic vesicle" usually shows cell-like structures similar to those of the pars prostatica although here they are more variable, sometimes almost filling the vesicle, sometimes few and scattered, or appearing as remnants of cells. Looss (1907, p. 140) interpreted these structures to be secretion droplets ("secrettropen"), noting that in *Sterrhurus* the vesicle may be lined with them; may show only scattered, isolated ones; may be free of them; or may contain sperm cells. We believe the entire absence of these cells (or droplets) is a generic character (see below). The function of the vesicle is quite unknown. In only two specimens, of hundreds examined, have we found sperm cells in the vesicle; their accumulation there is probably temporary or abnormal. On the other hand, the cell-like structures are usually very evident and are unlike secretion droplets in appearance, although if they are few in number some may have a distally bulged and proximally pointed shape. The membrane around them seems distinct, giving them the appearance of prostatic cells except that nuclei are lacking. If the cells are few and scattered, short fiber-like strands give the impression of frayed remnants of cell membranes. Looss explained the more empty appearance of some vesicles as resulting from coalescence of droplets. Study of living specimens might indicate whether these cell-like bodies are droplets of a secretion or not.

Croweroft (1946) proposed to distinguish *Sterrhurus* from *Lecithochirium* on the basis of an ejaculatory vesicle (free of the cells or droplets), or a prostatic vesicle containing such cells. One difficulty is to know the condition of the vesicle in the type species of these two genera. Looss states that the terminal ducts are similar in the two genera. His description of *S. musculus,* type species of *Sterrhurus,* indicates that the lining of the vesicle is a continuation of the ejaculatory duct, although he refers to it as "prostatic vesicle." He probably disregarded the "secrettropen" as a temporary, or at least inconstant, content of the vesicle and not a part of it. Manter (1947, p. 537) noted that *S. musculus* seemed to have a non-cellular lining to the vesicle. Jones (1943) restudied the type species of *Lecithochirium, L. rufoviride,* and indicated that the lining of the vesicle was a continuation of the pars prostatica. His figures show very small cells in the wall of the vesicle. The condition of the vesicle is probably the same in these two genera.

A complete clarification of these confusing data regarding the nature of the prostatic vesicle is not now possible. We propose: (1) That all valid species of both *Sterrhurus* and *Lecithochirium* possess a prostatic vesicle the inner wall of which is lined by transparent, cell-like structures which may sometimes disintegrate leaving only remnants; (2) that a distinct genus, *Separogermiductus* Skrj. & Gusch., 1955, is characterized by a large vesicle entirely free of cells or droplets and lined by a relatively thick, refractive wall (Fig. 6); (3) that the genera *Sterrhurus* and *Lecithochirium* be distinguished, as originally, by the absence or presence of a preacetabular pit.

Some of the species in the genera *Sterrhurus* and *Lecithochirium* are still difficult to delineate. The uterus and the ceca may or may not enter the ecsoma in most species. In our experience, the vitelline lobes are remarkably constant; for example, the short, stumpy lobes of the vitellaria of *S. flori-*

densis never have a length much greater than their width. Such vitellaria occur in *S. musculus, S. floridensis, S. microcercus, S. imocavus,* and *S. brevicirrus.* The terminal ducts of *S. brevicirrus* were not described; *S. imocavus* has a very large acetabulum; *S. microcercus* has a very short ecsoma. Several authors have suggested that *S. floridensis* is probably a synonym of *S. musculus.* Manter's comparison (1947) indicated that the only difference was the egg size (a maximum length of 17 microns in *S. floridensis* as compared with 19 to 21 microns in *S. musculus*). *S. floridensis* consistently has evident, cell-like bodies lining the prostatic vesicle, whereas these structures are not evident in *S. musculus.* However, as noted above, Looss probably considered these "secrettropen" to be transient bodies. There is some variation in them, particularly in specimens from *Lophius.* As these cells diminish the vesicle itself becomes larger so that the largest vesicles appear nearly empty. Looss did not state whether his egg measurements were from living specimens or not; if so, the difference in egg size would be explained. In any case, this single, rather small difference seems insufficient to justify *S. floridensis* and we consider it a synonym of *S. musculus.* Specimens from *Diplectrum formosum,* in particular, agree in all details (except egg size) with *S. musculus.*

The problem of identification of *S. floridensis* was discussed by Manter (1947, p. 346). Reexamination of specimens from various hosts continues to leave the impression that more than one species is represented but variations observed could not be correlated well enough to establish constant characters. Many specimens are probably progenetic and recently ingested. Even the smallest contained eggs; if these specimens are forms attained in the crustacean host, the fully developed adult may be somewhat different. Manter (1934) did not indicate the type host of *S. floridensis.* The first figure of the species was of a specimen from *Paralichthys* sp. and represents one of the *S. musculus* type. Specimens from *Diplectrum formosum* and at least some specimens from *Lophius* are also *S. musculus.*

The genera *Synaptobothrium* v. Linstow, 1904 and *Plerurus* Looss, 1907 are both characterized by the fact that the vitelline lobes are in two widely separated lateral groups. In both genera the sinus sac is represented only by parenchymal muscles. In fact, these two genera differ principally by the presence (in *Synaptobothrium*) or absence (in *Plerurus*) of a preacetabular pit. The vitelline lobes are longer in *Plerurus. Lecithochirium lobatum* Yam., 1952 from *Sphyraena* sp. and *Caranx* sp. from Celebes is clearly a synonym of *Plerurus digitatus* (Looss, 1899) Looss, 1907 from *Sphyraena vulgaris* from the Red Sea. *Lecithochirium longicaudatum* Yam., 1953 should be transferred to the genus *Plerurus,* becoming *P. longicaudatus* (Yam., 1953) n. comb. *Lecithochirium carangis* Yam., 1942 is also a *Plerurus* species, becoming *P. carangis* (Yam., 1942) n. comb.

The genus *Separogermiductus* was named by Skrjabin and Guschanskaja in 1955 with *Sterrhurus inimici* Yam., 1934 as type; also included were *St. musigarei* Yam., 1938, *St. magnus* Yam., 1938, and *St. pagrosomi,* Yam., 1939. In this genus the ejaculatory vesicle is large, globular, and conspicuous, being lined by a refractive, non-cellular wall, and free of cells. The pars prostatica enters this vesicle dorsally not far from its anterior end; the metraterm enters the sinus sac ventrally not far from the posterior end, then follows the surface of the vesicle ventrally and joins a very short hermaphroditic duct. The testes are largely extracecal. Yamaguti (1958) does not accept the genus *Separogermiductus.* He concluded that the terminal ducts of these various species all agreed with the type of *Sterrhurus* (*St. musculus*). It seems to us, however,

that the *Separogermiductus* condition of the terminal ducts (well illustrated in Crowcroft's (1946) Fig. 7 of *St. macrorchis*) is distinctly different from that in *St. musculus* where the pars prostatica enters the posterior end of the sinus sac and the vesicle has a lining of cells (or droplets). The Russian authors were incorrect in assuming the metraterm entered the vesicle; it actually enters the hermaphroditic duct at the anterior end of the vesicle as Yamaguti later (1938, p. 128) pointed out.

We accept the genus *Separogermiductus* for species of *Lecithochirium* or *Sterrhurus* (i.e. with or without a preacetabular pit) which have a bulbous ejaculatory vesicle, almost as large or even larger than the pharnyx, lined with a refractive non-cellular wall, empty of cells or droplets, and into which the pars prostatica enters dorsally and anteriorly. In addition to *Sep. inimici* (Yam., 1934), this genus will include *Sep. exodicus* (McFarlane, 1936) n. comb. (syn. *Lecithochirium exodicum*); *Sep. genypteri* (Manter, 1954) n. comb. (syn. *L. genypteri*); *Sep. macrorchis* (Crowcroft, 1946) n. comb. (syn. *St. macrorchis*); *Sep. magnatestis* (Park, 1936) n. comb., (syn. *St. magnatestis*); *Sep. magnus* (Yam., 1938) Skr. & Gusch., 1955; *Sep. musigarei* (Yam., 1938) Skr. & Gusch., 1955; *Sep. pagrosomi* (Yam., 1939) Skr. & Gusch., 1955; and a new species described below.

A study of specimens of *Sep. exodicus* collected by Hilda Ching at Friday Harbor, Washington, from the type host (ling cod) shows that the pars prostatica enters the ejaculatory duct near its anterior end (Fig. 4). *Sep. exodicus* differs from *Sep. magnatestis* only in having smaller testes. Measurements of 36 specimens confirm this difference.

Separogermiductus congeri, n. sp. (Figs. 5 and 6)

Host: *Conger cinereus* Ruppell, conger eel (Congridae), locally known as puhi uha (slippery eel) and puhi ula (red eel); 4 in 33 specimens examined.

Location: Intestine.

Holotype: U. S. Nat. Mus. Helminth. Coll., No. 39162.

Description (based on 4 specimens): Thick-bodied, 1.752 to 2.680 long by 0.584 to 0.871 wide; ecsoma very long but retracted, reaching halfway or more to acetabulum. Oral sucker 0.321 to 0.402 wide by 0.277 to 0.295 long, with a wide, bilobed papilla extending into lumen from anterodorsal wall. Acetabulum at midbody, 0.475 to 0.621 wide by 0.445 to 0.650 long, aperture typically elongate oval. Sucker ratio 1:1.4 to 1.6. Preacetabular pit a well-developed transverse groove with muscular anterior and posterior edges (Fig. 6). Pharynx 0.131 to 0.168 long by 0.110 to 0.161 wide; esophagus short; ceca not entering ecsoma but extending beyond its base. Forebody vesicular.

Testes symmetrical, wholly or mostly dorsal to posterior half of acetabulum, 0.219 to 0.322 long by 0.110 to 0.234 wide. Seminal vesicle preacetabular, tripartite, posterior part a rounded sac, middle part slightly elongate, anterior part smallest and narrowing to a pars prostatica which enters base of sinus sac. Within the sac, pars prostatica enlarges to form an elongate, pyriform prostatic vesicle lying between wall of sinus sac and ejaculatory vesicle; from

All figures drawn with the aid of a camera lucida. The projected scale has its value indicated in millimeters. Abbreviations used: *ce,* cecum; *ejv,* ejaculatory vesicle; *ex,* excretory system; *ga,* genital atrium, *gp,* genital pore, *hd,* hermaphroditic duct; *mt,* metraterm; *pa,* preacetabular pit; *pp,* pars prostatica; *prg,* prostatic gland cells; *prv,* prostatic vesicle; *sph,* sphincter muscle; *ss,* sinus sac; *sv,* seminal vesicle; *ut,* uterus.

PLATE I

Fig. 1. *Plicatrium lycodontis* (Myers and Wolfgang, 1953) from *Lycodontis* sp., New Hebrides; holotype; ventral view of terminal genital ducts with genital atrium everted.

Fig. 2. *P. lycodontis,* paratype; lateral view of terminal genital ducts, genital atrium contained in sinus sac.

Fig. 3. *P. lycodontis,* paratype; ventral view of vitellaria.

Fig. 4. *Separogermiductus exodicus* (McFarlane, 1936) from *Ophiodon elongatus* (type host) San Juan Island, Washington; ventral view of terminal genital ducts.

Fig. 5. *Separogermiductus congeri* from *Conger cinereus,* holotype; dorsal view.

Fig. 6. *S. congeri,* paratype; ventral view of terminal genital ducts.

Fig. 7. *Lecithochirium microstomum* Chandler, 1935 from *Chaetodon miliaris,* ventral view.

Fig. 8. *L. microstomum* from *C. miliaris,* ventral view of terminal genital ducts.

Fig. 9. *Lecithochirium synodi* Manter, 1931 from *Synodus foetens,* Beaufort, North Carolina; paratype; ventral view of forebody showing preacetabular pit.

Fig. 10. *Sterrhurus goslinei* from *Conger cinereus,* holotype; ventral view.

Fig. 11. *St. goslinei,* (a) ventral view of terminal genital ducts; (b) diagram of seminal vesicle, pars prostatica, and prostatic vesicle.

Fig. 12. *St. goslinei,* ventral view of vitellaria.

the prostatic vesicle a duct leads anteriorly and penetrates dorsal wall of ejaculatory vesicle through a funnel-like tube (Fig. 6); prostatic gland cells around pars prostatica outside sinus sac. Ejaculatory vesicle rounded, almost as large as pharynx, opening directly into base of a short, wide, muscular hermaphroditic duct. Genital pore median or submedian at level of pharynx or esophagus.

Ovary immediately postacetabular, subtriangular, 0.088 to 0.183 long by 0.146 to 0.263 wide, submedian (right in 3 specimens, left in 1). Vitellaria immediately postovarian, consisting of 7 distinct, digitate lobes narrowed and joined at bases. Uterus may or may not extend posterior to vitellaria, not entering ecsoma, becoming a muscular metraterm at anterior edge of acetabulum; metraterm entering base of sinus sac within which it becomes a thin-walled tube between ejaculatory vesicle and ventral wall of sinus sac, entering hermaphroditic duct at anterior end of vesicle. Seminal receptacle not observed. Eggs 17 to 19 by 8 to 11 microns.

Comparisons: This species differs from all others in the genus in that the ecsoma reaches halfway or more to the acetabulum and the testes are mostly dorsal to the acetabulum. The vitelline lobes are much shorter in *Sep. magnatestis, Sep. exodicus, Sep. musigarei,* and *Sep. genypteri.* The oral sucker is relatively smaller in all species except *Sep. magnus.*

Lecithochirium microstomum Chandler, 1935 (Fig. 7-8)
Synonym: *L. sinaloense* Bravo-Hollis, 1956

HOST: *Pseudupeneus multifasciatus* (Quoy and Gaimard), locally known as moana or moano, the red and black banded goatfish (Mullidae); 1 specimen from 1 of 20 hosts. New host record.

DISCUSSION: This species has been reported from the Gulf of Mexico and the Galapagos Islands. Chandler's type specimens were from *Trichiurus lepturus,* cutlass fish, in Galveston Bay. Manter (1940) reported the species from four kinds of fishes in the Galapagos Islands and later (1947) from three species at Tortugas, Florida. The record from *Ancylopsetta dilecta,* a flounder from 100 fathoms at Tortugas, is a doubtful one; a preacetabular pit cannot be observed on this specimen and the vitelline lobes are a little longer than usual for the species. Sogandares-Bernal and Hutton (1959) report *L. microstomum* from *Coryphaena equisetis* from near Tampa, Florida There seems little doubt that the Galapagos material is like the Hawaiian specimen and no important differences between them and Chandler's can be detected.

This species is characterized by a preacetabular pit, either non-glandular or with inconspicuous gland cells; sucker ratio of 1:2.5 to 2.8; vitelline lobes about the same length as width or slightly longer, up to twice as long; sinus sac rather broad, cylindrical to pyriform, containing a spherical prostatic vesicle, wide and usually short, muscular hermaphroditic duct, and short wide genital atrium. The holotype specimen, kindly loaned from the National Museum Helminthological Collection, has a contracted forebody so that the sinus sac appears amost as wide as long. Linton's figure of a sagittal section seems to show an open type sinus sac but following sections clearly show a complete pyriform sac.

L. synodi is a related species with sucker ratio extending up to 2.5. A restudy of paratypes from *Synodus foetens* from Beaufort, N. C., shows that the preacetabular pit is not only highly glandular but that it has circular muscles (Fig. 9). Its vitelline lobes are somewhat longer than in *L. microstomum.*

The single specimen of *L. sinaloense* Bravo-Hollis, 1956 from *Muraenasox coniceps* from the Mexican Pacific agrees with *L. microstomum* in most respects except that one vitellarium appears to have only two lobes. We believe this appearance may be due to angle of vision or to an abnormality. The egg size of *L. sinaloense* (20 to 23 by 12 to 17 microns) has a large maximum width. However, the thin-shelled eggs of this genus vary considerably in form. We can extend Chandler's record (16 by 12 microns) to 16 to 19 by 11 to 12 microns in the holotype. The following measurements (in microns) are those of favorable eggs, each in *L. microstomum* from a different host: 22 by 11 to 13; 19 to 20 by 11 to 12; 21 by 11; 22 by 13; 24 by 13.

Sterrhurus goslinei, n. sp. (Fig. 10-12)

HOST: *Conger cinereus* Ruppell, conger eel (Congridae), locally known as puhi uha (slippery eel) and puhi ula (red eel); 11 specimens from 35 hosts.

LOCATION: Intestine.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39163.

DESCRIPTION (based on 11 specimens): Body proper 1.708 to 3.030; total length 1.708 to 3.562 including ecsoma (extended from none to 1.0), greatest width either anterior to acetabulum or at level of ovary, 0.511 to 0.918. Forebody unusually muscular, sides often curved ventrally; preacetabular pit absent but concavity formed by distinct muscle fibers present. Parenchyma vesicular. Preoral lobe well developed, often drawn ventrally toward mouth but sometimes elevated to form a trough toward mouth; oral sucker 0.183 to 0.275 wide by 0.161 to 0.235 long; acetabulum in anterior half of body, aperture a longitudinal slit, 0.292 to 0.504 wide by 0.380 to 0.548 long; sucker ratio 1:1.6 to 1.9. Pharynx 0.080 to 0.131 long by 0.102 to 0.127 wide; esophagus short; ceca relatively wide, extending to near posterior end of body where they narrow abruptly and enter ecsoma.

Testes immediately postacetabular, symmetrical, contiguous, one on either side of median line, usually transversely elongate, 0.095 to 0.208 long by 0.131 to 0.255 wide; seminal vesicle bipartite, preacetabular or overlapping as much as anterior fourth of acetabulum; posterior part thin-walled, elongate, saccular; anterior part thick-walled, of similar shape, tapering to a thin-walled portion which forms a loop before joining pars porstatica by a short, narrow duct; pars prostatica bends to enter sinus sac; prostatic cells surrounding pars prostatica near sinus sac; sinus sac pyriform, containing cylindrical prostatic vesicle and prostatic cells. Hermaphroditic duct partially protrusible. Genital pore median, ventral to pharynx or espohagus (Fig. 11).

Ovary subspherical, 0.117 to 0.221 long by 0.183 to 0.277 wide, median or submedian, near middle or a little anterior to middle of hindbody; seminal receptacle lacking; 7 vitelline lobes, digitate, considerably longer than wide, connected by narrow bases, some forked at tip (Fig. 12). Uterus extends to near posterior end of body; well-developed sphincter muscle between uterus and metraterm; metraterm ventral to middle part of seminal vesicle, entering sinus sac posteriorly. Eggs 13 to 18 by 8 to 9 microns.

The species is named for Dr. William E. Gosline, ichthyologist, who identified the fish hosts of this collection.

DISCUSSION: This species differs from all others in the genus by the longitudinal aperture of the acetabulum together with a preacetabular concavity bordered by conspicuous muscles. It resembles *S. pacificus* (Yam., 1942) Yam., 1958, particularly in the long vitelline lobes, some of which are subdivided, the symmetrical testes, sphincter at base of the metraterm, and thickened wall

of the anterior part of the seminal vesicle. *S. goslinei* has smaller eggs (13 to 18 microns long compared with 21 to 24); its acetabulum is smaller while the oral sucker is larger, making the sucker ratio 1:1.6 to 1.9 compared with 1:2.4 to 3.0; the seminal vesicle is more distinctly divided.

Elytrophallus mexicanus Manter, 1940 (Figs. 13-14)

HOSTS: *Pseudupeneus multifasciatus* (Quoy and Gaimard), locally known as moana or moano, the red and black banded goatfish (Mullidae); 1 each from 4 of 20 hosts. New host record.

P. chrysoneus Jordan and Evermann, a goatfish (Mullidae); 1 from 1 of 23 hosts. New host record.

LOCATION: Intestine.

DISCUSSION: These specimens agree in general with the original description. A few differences are: the sucker ratio is 1:2.4 to 2.5 as compared with 1:3; the loop from the seminal vesicle extends as far back as the posterior testis; the hermaphroditic duct may be sinuous dorsal to the acetabulum and does not become extensively coiled in any of the 5 specimens; the distal end of the duct lies free in the sinus sac or may extend into the atrium; the genital atrium is muscular most of its length but is thin-walled near genital pore (Fig. 13); eggs are slightly smaller, 11 to 14 by 6 microns.

A restudy of 25 paratypes in Manter's collection disclosed that the differences in the terminal ducts noted above also appeared in some of the paratypes. Specimens from all localities bear a pair of papillae on the preoral lobe (Fig. 13). As Yamaguti (1958) has noted, the label "ms" in Manter's (1940) Fig. 106 refers to a muscular portion of a long, tubular genital atrium, not to a "metraterm sac."

This species is known from 7 different hosts along the Mexican Coast and Galapagos, from *Paralabrax clathratus* at LaJolla, California, as well as from goatfishes in Hawaii.

Skrjabin and Guschanskaja (1954) named the family Elytrophallidae for this genus, basing it largely on the terminal genital ducts. Although the hermaphroditic duct is long and lies free in its sac, the system is basically a sinus sac enclosing a tubular hermaphroditic duct and connecting with a genital atrium. We believe the genus may be included in the Lecithochiriinae.

Musculovesicula bilabiata, n. sp. (Figs. 15-19)

HOST: *Conger cinereus* Ruppell, conger eel (Congridae), locally known as puhi uha (slippery eel) and puhi ula (red eel); 38 specimens in 13 hosts.

LOCATION: Intestine.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39161.

DESCRIPTION (based on 7 specimens): Body 2.088 to 3.103 by 0.767 to 0.933; both ends slightly tapered and truncate; ecsoma retracted in all 38 specimens. Oral sucker 0.270 to 0.365 wide by 0.219 to 0.307 long, subterminal, embedded in body; two large dorso-lateral preoral lobes, each with an irregularly shaped pad on ventral surface; pads may extend longitudinally on dorsal wall of oral cavity or may be everted (Figs. 17-18). Acetabulum large, 0.686 to 0.774 wide by 0.613 to 0.796 long, occupying second fourth of body length; aperture longitudinal oval; sucker ratio 1:2.1 to 2.8. Pharynx 0.117 to 0.153 long by 0.146 to 0.163 wide; esophagus very short; ceca sinuous, extending to last fourth of body.

Testes oval, symmetrical, far apart, lateral, dorsal to posterior half of acetabulum, 0.110 to 0.270 long by 0.110 to 0.219 wide. Seminal vesicle lying

PLATE II

Fig. 13. *Elytrophallus mexicanus* Manter, 1940 from *Pseudupeneus multifasciatus*, ventral view.

Fig. 14. *E. mexicanus*, dorsal view of terminal genital ducts.

Fig. 15. *Musculovesicula bilabiata* from *Conger cinereus*, holotype; ventral view.

Fig. 16. *M. bilabiata*, ventral view of terminal genital ducts.

Fig. 17. *M. bilabiata*, paratype; oral pads partially everted (terminal genital ducts omitted).

Fig. 18. *M. bilabiata*, paratype; oral pads entirely everted (terminal genital ducts omitted).

Fig. 19. *M. bilabiata*, eggs.

Fig. 20. *Sterrhurus praeclarus* Manter, 1934 from *Merluccius* sp., holotype; ventral view of terminal genital ducts.

Fig. 21. *Hysterolecitha tinkeri* from *Pomacentrus inornatus*, holotype; ventral view.

Fig. 22. *H. tinkeri*, paratype; lateral view of terminal genital ducts.

Fig. 23. *H. tinkeri*, eggs.

Fig. 24. *Genolinea ampladena* from *Acanthurus olivaceus*, holotype; ventral view.

Fig. 25. *G. ampladena*, ventral view of terminal genital ducts.

Fig. 26. *G. ampladena*, Mehlis' gland.

more or less transversely in preacetabular region, 0.475 to 0.693 long by 0.168 to 0.302 wide, with wall of circular muscles 0.032 to 0.067 thick. Short prostatic duct arises from anterior end of seminal vesicle, enters sinus sac and unites with metraterm to form hermaphroditic duct; hermaphroditic duct first a short, narrow, thin-walled duct, then an expanded, thick-walled chamber, and finally a tubular thin-walled portion; prostatic cells not observed. Sinus sac thick-walled, 0.110 to 0.224 long by 0.077 to 0.183 wide, partially protrusible. Genital pore median ventral to oral sucker.

Ovary rounded, 0.117 to 0.204 long by 0.131 to 0.263 wide, not far posterior to acetabulum, submedian (left in holotype and about half of specimens, right in others). Seminal receptacle lacking. Vitelline lobes 7, digitate, lobes several times longer than wide, sinuous, more or less radially arranged at ventral posterior edge of ovary. Uterus coils to near tips of ceca then forward median to ovary and between testes; conspicuous sphincter muscle at distal end of uterus; metraterm parallels seminal vesicle ventrally, joins prostatic duct at base of sinus sac. Eggs 13 to 19 by 8 to 9 microns, with unipolar filament nearly as long as egg (Fig. 19). Excretory crura join dorsal to pharynx.

DISCUSSION: *Musculovesicula gymnothoracis* Yam., 1940, type of the genus, is from an eel, *Gymnothorax kidako*, in Japan. *M. bilabiata* differs from it in the preoral and oral lobation, more anterior genital pore, more transverse seminal vesicle, filamented and smaller eggs. Although a filamented egg is sometimes considered a generic character, the filaments here are inconspicuous, and it is possible they were overlooked in the type species.

Skrjabin and Guschanskaja (1955) transferred *Sterrhurus praeclarus* Manter, 1934 to *Musculovesicula*, apparently on the basis of the short sinus sac, symmetrical testes, and weakly developed ecsoma. The absence of the muscular seminal vesicle characteristic of *Musculovesicula* seems to have been overlooked. A restudy of the type specimen of *S. praeclarus* shows that the "sinus sac" is atypical, consisting of semi-circular muscle fibers which diverge anteriorly (Fig. 20). We prefer to retain the species in *Sterrhurus* for the present.

OTHER SUBFAMILIES OF HERMIURIDAE

Subfamilies of Hemiuridae lacking a "tail" or ecsoma are Aphanurinae, Prosorchiinae, and Lecithasterinae (synonym Derogenetinae). The first two are very small subfamilies, but the Lecithasterinae is a large subfamily Some authors separate the Derogenetinae (with compact vitellaria) from the Lecithasterinae, but gradations in the lobing of the vitellaria make it difficult to recognize two subfamilies here.

Subfamily Lecithasterinae Odhner, 1905

This subfamily includes hemiurids without ecsoma, without cuticular plications, with paired vitellaria which may be unlobed, lobed, or digitate, or the lobes may become separated into seven parts; the testes are not preacetabular.

Pigulewsky (1938) established the genus *Mordvilkoviaster* for *Lecithaster galeatus* Looss, 1907 on the basis of a globular (unlobed) ovary. Looss himself was very uncertain regarding this character; he omits the ovary in his figure and states it was too inconspicuous to determine its shape. We consider *Mordvilkoviaster* a synonym of *Lecithaster*. *M. anisotrema* (MacCallum, 1921) belongs in the genus *Brachadena* and is a synonym of *B. pyriformis* Linton, 1910, as indicated by Manter (1931, p. 409).

The genus *Intuscirrus* Acena, 1947 seems clearly to be a synonym of *Genolinea* Manter, 1925. *I. aspicotti* is probably a synonym of either *G. manteri* Lloyd, 1938 or *G. laticauda* Manter, 1925.

The above concept of Lecithasterinae would include the following 30 genera: *Aponurus* Looss, 1907; *Arnola* Strand, 1942; *Brachadena* Linton, 1910; *Bunocotyle* Odhner, 1928; *Derogenes* Lühe, 1900; *Derogenoides* Nicoll, 1912; *Dictysarca* Linton, 1910; *Genarchopsis* Ozaki, 1925 (syn. *Progonus* Looss, 1899; *Genarches* Looss, 1902; *Ophiocorchis* Srivastava, 1933); *Genolinea* Manter, 1925 (syn. *Parasterrhurus* Manter, 1934; *Intuscirrus* Acena, 1947); *Gonocerca* Manter, 1925; *Gonocercella* Manter, 1940; *Halipegus* Looss, 1899 (syn. *Genarchella* Travassos, Artigas et Pereira, 1928); *Hemipera* Nicoll, 1912 (syn. *Hemiperina* Manter, 1934); *Hypohepaticola* Yam., 1934; *Hysterolecitha* Linton, 1910; *Hysterolecithoides* Yam., 1934; *Indoderogenes* Srivastava, 1931; *Lampritrema* Yam., 1940; *Lecithaster* Lühe, 1901 (syn. *Dichadena* Linton, 1910; *Mordvilkoviaster* Pigulewsky, 1938); *Lecithophyllum* Odhner, 1905; *Leurodera* Linton, 1910; *Liopyge,* Looss, 1899 (syn. *Liocerca* Looss, 1902); *Macradena* Linton, 1910; *Macradenina* Manter, 1947; *Mitrostoma* Manter, 1954; *Opisthadena* Linton, 1910; *Pronopyge* Looss, 1899; *Tangiopsis* Skr. & Gusch., 1955; *Theletrum* Linton, 1910; *Trifoliovarium* Yam., 1940; *Vitellotrema* Guberlet, 1928.

Hysterolecitha tinkeri, n. sp. (Figs. 21-23)

HOSTS: *Pomacentrus inornatus* DeVis, damsel fish (Pomacentridae); 17 from 28 hosts examined. *Abudefduf abdominalis* (Quoy & Gaimard), locally known as maomao (Pomacentridae); 1 from 1 of 8 hosts examined.

LOCATION: Intestine.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39160.

DESCRIPTION (based on 18 specimens): Body 2.077 to 4.389 long, 0.248 to 0.469 wide where body enlarges at level of acetabulum; forebody 0.402 to 0.938 long or about ⅕ to ¼ total length, sides not tapering, anterior end of body rounded; hindbody with nearly parallel sides, posterior end of body rounded. Mouth subterminal with small preoral lobe; oral sucker 0.141 to 0.194 wide by 0.127 to 0.221 long; acetabulum protuberant, 0.241 to 0.402 wide by 0.241 to 0.436 long; sucker ratio 1:1.7 to 2.0. Pharynx rounded, 0.054 to 0.101 long by 0.059 to 0.094 wide, sometimes pushed anteriorly into oral sucker as much as half its length; esophagus 0.024 to 0.067 long; ceca extending to posterior end of body.

Testes tandem to slightly oblique, anterior testis 0.101 to 0.201 long by 0.128 to 0.168 wide, posterior testis 0.114 to 0.174 long by 0.114 to 0.174 wide, anterior testis 0.335 to 1.106 behind acetabulum ($^1/_7$ to $^1/_4$ body length), testes separated 0.047 to 0.168 by coils of uterus; seminal vesicle tubular, 0.160 to 0.214 long, preacetabular or overlapping only anterior border of acetabulum; pars prostatica 0.080 to 0.168 long surrounded by prostatic cells, entering posterior end of sinus sac; sinus sac intercecal, 0.127 to 0.248 (0.280 in 1 specimen) long by 0.067 to 0.150 wide surrounding a wide, muscular, partially eversible hermaphroditic duct; genital pore large, median, immediately posterior to cecal bifurcation.

Ovary rounded, 0.101 to 0.188 long by 0.128 to 0.235 wide, median, in posterior part of hindbody, 0.200 to 0.536 behind posterior testis; seminal receptacle not observed; 7 digitate vitelline lobes joined at base immediately posterior to ovary, 0.235 to 0.442 long and wider than body because two an-

terior lobes on each side extend laterally and dorsally, three median lobes extend posteriorly. Uterus descends to near end of body, then ascends to level of acetabulum in large loops dorsally and between the gonads, enters posterior end of sinus sac, immediately joining pars prostatica to form hermaphroditic duct. Eggs ovate, operculate, 21 to 27 by 10 to 12 microns with bipolar filaments about twice the length of egg.

Excretory pore terminal, vesicle tubular, giving off a pair of arms that meet dorsal to pharynx; granular material present in arms.

The species is named for Spencer W. Tinker, Director of the Waikiki Aquarium, Honolulu. Mr. Tinker assisted the collection of trematodes by supplying many of the fish hosts.

DISCUSSION: None of the 10 other species of *Hysterolecitha* Linton, 1910 is described as having filamented eggs. The filamented condition might be overlooked if the eggs are crowded in the uterus. However, examination of specimens of *H. rosea* Linton, 1910 (type species of the genus) and *H. elongata* Manter, 1931, confirms the absence of filaments in these species.

In other respects, *H. tinkeri* resembles *H. microrchis* Yam., 1934, *H. xesuri* Yam., 1938, and *H. nahaensis* Yam., 1942. It differs from all of them by (1) longer, more slender body, (2) more anterior acetabulum, (3) tubular, untwisted seminal vesicle (as compared with saccular, twisted, or winding), and (4) longer pars prostatica. In addition, it differs from *H. microrchis* in larger testes and ovary, larger sinus sac, somewhat smaller sucker ratio, and more posterior extent of the uterus; from *H. nahaensis* in larger gonads, much larger sinus sac, consistently 7 vitelline lobes, and absence of a seminal receptacle; from *H. xesuri* by slightly smaller sucker ratio.

A study of sagittal sections of a paratype of *H. elongata* shows its terminal ducts similar to those of other species but weakly developed. The small sinus sac surrounds only the hermaphroditic duct; prostatic cells are reduced to a few small cells around the tubular pars prostatica; the genital pore is very inconspicuous ventral to the pharynx.

Genolinea ampladena, n. sp. (Figs. 24-26)

HOST: *Acanthurus olivaceus* Bloch and Schneider, locally known as naenae or orange spot tang (Acanthuridae); 6 specimens from 2 of 7 hosts examined.

LOCATION: Stomach.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39158.

DESCRIPTION (based on 6 specimens): Body elongate, more or less cylindrical, 1.112 to 1.407 by 0.208 to 0.302, widest at level of acetabulum, each end bluntly rounded. A transverse cuticular line extends across the body ventrally just posterior to acetabulum in three specimens; not visible in other three specimens. Oral sucker ovate, retracted into body, 0.072 to 0.104 wide by 0.074 to 0.120 long; body wall forms a rim around the sucker; rim thickened dorsally. Acetabulum about ⅓ body length from anterior end, sometimes just anterior to midbody; 0.160 to 0.227 wide by 0.168 to 0.240 long; sucker ratio 1:2 to 2.7; aperture transverse. Transverse muscle fibers in both anterior and posterior lips of acetabulum, better developed in posterior lip; sphincter muscle absent. Pharynx 0.032 to 0.048 long by 0.043 to 0.061 wide; esophagus 0.016 to 0.054 long; ceca rather narrow, ending blindly near posterior end of body.

Testes tandem, contiguous, just anterior to middle of hind-body, separated from acetabulum by uterus. Seminal vesicle preacetabular or overlapping

only anterior edge of acetabulum, to left of midline, a short wide tube bent sharply near middle. Prostatic vesicle intercecal, large, ovoid, 0.064 to 0.086 long by 0.040 to 0.069 wide, surrounded by a single layer (double in one specimen) of large cuboidal cells; pars prostatica a short, wide duct which joins metraterm at base of sinus sac, surrounded by a few small gland cells. Sinus sac pyriform, well developed, 0.067 to 0.080 long by 0.056 to 0.064 wide; basal third of hermaphroditic tube swollen, sac-like, thin-walled; distal two thirds a muscular tube opening on a cone-shaped elevation partially protrusible through genital pore; only a few small gland cells in sinus sac. Almost all species of *Genolinea* show this bipartite condition of the hermaphroditic tube.

Ovary rounded to transversely oval; 0.048 to 0.080 long by 0.067 to 0.083 wide, posterior to middle of hindbody; Laurer's canal present; seminal receptacle dorsal and to the left or right of ovary, almost as large as ovary, 0.056 to 0.083 long by 0.0040 to 0.067 wide. Vitellaria two compact masses immediately postovarian; usually obliquely tandem, tandem in one specimen, almost symmetrical in holotype. Uterus extends posterior to vitellaria to near posterior end of body, coils between ovary and testes, and between testes and acetabulum; metraterm short or nearly as long as prostatic vesicle, with longitudinal muscles. Eggs yellowish, relatively large, 30 to 40 by 14 to 21 microns; those in proximal portion of uterus distinctly smaller than those near sinus sac.

Excretory vessels join dorsal to pharynx.

The name *ampladena* is derived from *amplus* (= large) and *adenos* (= gland) and refers to the large prostatic cells surrounding the prostatic vesicle.

DISCUSSION: Although the transverse marking across the body posterior to the acetabulum suggests the genera *Stomachicola, Theletrum* or *Opisthadena,* it is not visible on three specimens and should not be considered as demarking an ecsoma. Another variable character is the tandem, oblique, or symmetrical position of the vitellaria. The species differs from all others in the genus in its large ovate prostatic vesicle surrounded by a single layer of large cuboidal cells. Another distinctive character is the wide acetabulum with narrow, transverse aperture. The body size is smaller than in other species except *G. oncorhynchi* Margolis and Adams, 1956, which is probably the nearest related species. *G. oncorhynchi* has a sphincter in the acetabulum and smaller eggs.

SUMMARY

1. The families and subfamilies of the Hemiuroidea are discussed briefly.

2. A new genus, *Plicatrium,* is named for *Lecithochirium lycodontis* Myers and Wolfgang, 1953.

3. The genus *Separogermiductus* Skrjabin and Guschanskaja, 1955 is recognized on the basis of its large, smooth-walled ejaculatory vesicle.

4. The following synonyms are proposed: *Sterrhurus floridensis* Manter, 1947, syn. (at least in part) of *S. musculus* Looss, 1907; *Lecithochirium lobatum* Yamaguti, 1952, syn. of *Plerurus digitatus* (Looss, 1907); *L. sinaloense* Bravo-Hollis, 1956, syn. of *L. microstomum* Chandler, 1935.

5. The following new combinations are proposed: *Plerurus longicaudatus* (Yam., 1953) (formerly *Lecithochirium*); *P. carangis* (Yam., 1942) (formerly *Lecithochirium*); *Separogermiductus exodicus* (McFarlane, 1936) (formerly *Lecithochirium*); *Sep. genypteri* (Manter, 1954) (formerly *Lecithochirium*); *Sep. macrorchis* (Crowcroft, 1946) (formerly *Sterrhurus*); *Sep. magnatestis* (Park. 1936) (formerly *Sterrhurus*).

6. The following new species are named: *Separogermiductus congeri, Sterrhurus goslinei, Musculovesicula bilabiata, Hysterolecitha tinkeri, Genolinea ampladena.*

7. Two species are also known elsewhere: *Lecithochirium microstomum* Chandler, 1935 and *Elytrophallus mexicanus* Manter, 1940.

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Three New Nasal Mites (Acarina: Speleognathidae) from the Gray Squirrel, the Common Grackle, and the Meadowlark in the United States

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Three new speleognathid nasal mites were studied at the Patuxent Research Refuge during 1958-1959 in the course of investigations on the parasites of native birds and mammals. It is possible that at least one of these mites can serve as a disease vector. This species, commonly found in the turbinates of the eastern meadowlark, apparently ingests whole blood. After feeding, it becomes a bright red color which darkens, as digestion progresses, to a dark brown or black in a few days. This type of mite, intimately associated with the mucus membranes and probably spread from parent to young in the feeding process, has considerable potential as a disease vector.

The first speleognathid found in a mammal in this county is described in this paper. As these mites have been reported from a number of mammals (bats, rodents, cows) in other parts of the world, it is likely that more will be found here. The three species herein described brings the known species from the western hemisphere to a total of eight.

Fain (1958b) has established a number of sub-genera in the Speleognathidae without changing the existing generic concepts. As other interpretations of speleognathid taxonomy seem equally plausible this paper will not employ these sub-genera.

All measurements in the following descriptions are in millimeters.

Speleognathopsis sciuri, n. sp.

ADULT: (Figs. 1, 5) Milky white in color; elongate oval in shape with the greatest width in the humeral region. Length, excluding gnathosoma, 0.40; width, 0.26 at the humeral region. Gnathosoma directed ventrally, about 0.045 long by 0.06 wide at base. Palpi with three segments, the proximal segment weak and poorly demarcated; palpal tarsus directed ventrally beneath the tibia; palpal tibia provided with one short, ciliated, barbelled seta apically; palpal tarsus with what are apparently three short, barbelled and one nude, sensory setae ventrally (the details of these setae are not clear). Cheliceral bases provided with two pairs of short, expanded, barbelled setae. Gnathosomal base with heavy reticulations; gnathosomal cuticle with fine linear striations and reticulations.

DORSUM: Eyes lacking; reticulate scutum present with enclosed inner cell holding a pair of dorsal setae (Fig. 5). Anteriorally, with a pair of elongate, 0.022 long, slightly expanded, barbelled sensillae (Fig. 11b) set in deep pseudostigmata and a pair of short, expanded, barbelled, presensillar setae. Dorsal formula 4-4-2-2-4, composed of short, ciliated, barbelled setae. Dorsal cuticle with fine striations containing linear punctations.

VENTER: With three pairs of short, ciliated, barbelled sternal setae, three pairs of similar but smaller genital setae, and three pairs of larger ciliated, barbelled anal setae. Genital plates lacking; genital slit about 0.045 long with vague anterior, subsurface reticulations. Anal area also without plates. Coxae in two groups, anterior and posterior, with heavy, subsurface, supporting reticulations. Distance between coxae I, 0.027; between coxa II and coxa III, 0.087. Ventral cuticle with faint striations and punctations.

LEGS: With strong supporting reticulations and transverse bands; cuticle with fine longitudinal striations and punctations. Leg setae all of an elongate, ciliated, barbelled type (Fig. 11a), with the exception of the tarsal setae which are nude, sensory; widely expanded, barbelled; or moderately expanded, elongate, barbelled setae with short, blunt bayonets. Arrangement of leg setae as follows:

Coxae: I = 2; II = 1; III = 1; IV = 1;
Trochanters: I = 1; II = 1; III = 0; IV = 0;
Femurs: I = 4; II = 3; III = 3; IV = 1;
Genus: I = 4; II = 4; III = 3; IV = 3;
Tibiae: I = 4; II = 2; III = 2; IV = 2;
Tarsi: I = 14; dorsally, with one nude sensory seta; four elongate, slightly expanded, barbelled setae with short, blunt bayonets; one highly expanded barbelled seta with bayonet; and two moderately elongate, expanded, ciliated, barbelled setae; ventrally, with two expanded, barbelled setae and four ciliated, barbelled setae; II = 9, dorsally, with one nude sensory seta; one large expanded, barbelled, seta; two elongate, expanded, ciliated, barbelled setae; and two moderately expanded, elongate, ciliated, barbelled setae; ventrally, with three expanded barbelled setae; III = 7, dorsally, with four enlarged, ciliated, barbelled setae; ventrally, with three expanded, ciliated, barbelled setae; IV = 7, dorsally, with one large, expanded, barbelled and three expanded, ciliated, barbelled setae; ventrally, with three expanded, barbelled setae. Pulvillus of all legs entire and setated; all claws strong and normal.

Only one specimen of this mite is now available. The characters closely agree with those of *Speleognathopsis strandtmanni* Fain 1955, which was described from a squirrel, *Funisciurus carruthersi* Thom. in the forests of Bururi (Urundi, Africa) at an altitude of 2200 m. (Fain, 1956).

DIAGNOSIS: *S. sciuri* may be distinguished from all other known species of the genus *Speleognathopsis* by the configuration of the dorsal scutum and the form of the sensillae. It may be distinguished particularly from *S. strandtmanni* by the character of the sensillae, which in *strandtmanni* are simple, divided apically into three fine branches, whereas in *sciuri* they are slightly expanded and completely barbelled.

HOLOTYPE: Adult U.S.N.M. #2563 is deposited in the collection of the United States National Museum, Washington, D. C.

TYPE HOST: *Sciurus carolinensis* Gmelin, 1788, the common gray squirrel of the United States.

TYPE LOCALITY: Patuxent Research Refuge, Laurel, Maryland.

Boydaia sturnellae, n. sp.

ADULT: (Figs. 2, 10) Bright red through brown to blackish in color; elongate oval in shape, with the greatest width toward the middle of the body; length, excluding gnathosoma, 0.46; width, 0.31.

GNATHOSOMA: Width at base 0.08; length from base to apex 0.72; cheliceral bases bearing two pairs of short, expanded, barbelled setae; gnathosomal base with strong supporting reticulations; cuticle with minute reticulations at the surface. Palpi three segmented, approximately 0.036 long; palpal tarsus about 0.01 long, bearing three short, barbelled setae, one apically and two ventrally; palpal tibia about 0.02 in length.

DORSUM: Eyes and scutum lacking. Anteriorally, with a pair of thin, barbelled sensillae 0.027 long and a short pair of presensillar setae. Dorsal

setae barbelled, slightly expanded, about 0.009 long. Dorsal formula 4-4-2-2-4. Cuticle finely striate and punctate.

VENTER: With three pairs of sternal setae, two pairs of pre-genital setae, three pairs of genital setae, and two pairs of anal setae, all short and barbelled. Abdomen with a long, folded genital slit about 0.06 long. Anus 0.018 long surrounded by three very weak plates. Ventral cuticle finely striated and punctate.

LEGS: Well developed and sclerotized, with strong internal reticulations and cuticular striations and punctations. Cuticle also with minute reticula-

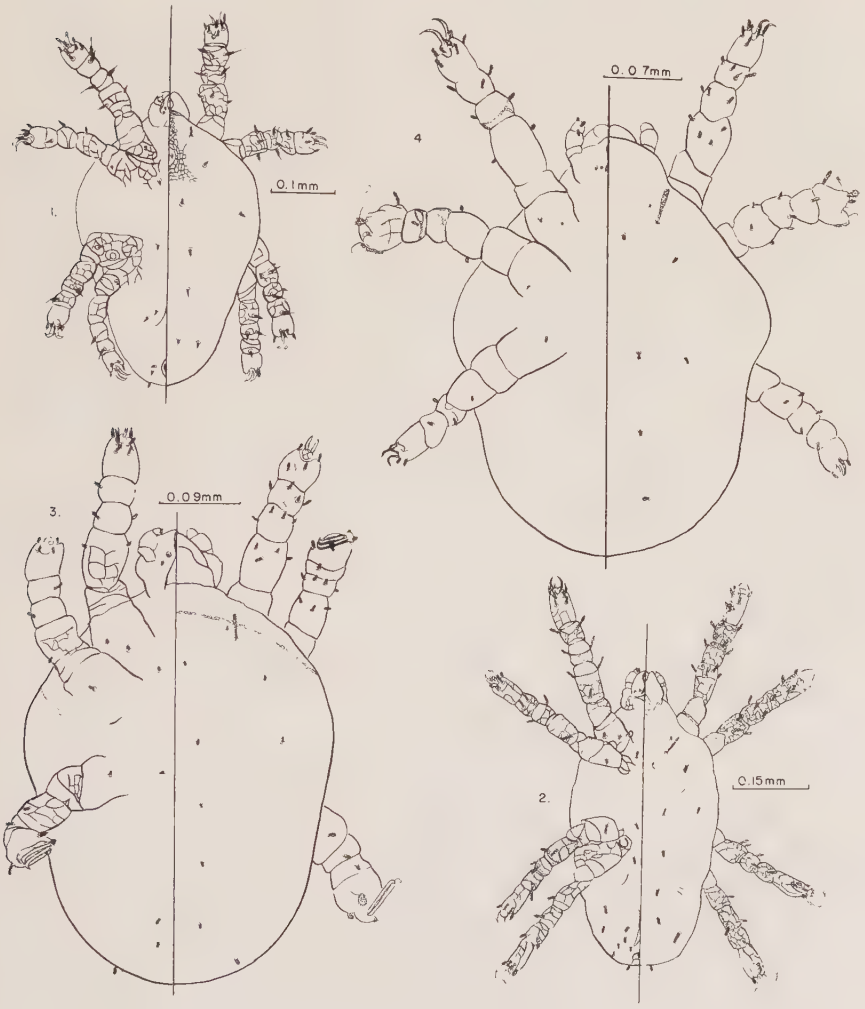

Figure 1. *Speleognathopsis sciuri,* adult. Ventral and dorsal aspects; Figure 2. *Boydaia sturnellae,* adult. Ventral and dorsal aspects; Figure 3. *Boydaia sturnellae,* larva. Ventral and dorsal aspects; Figure 4. *Boydaia quiscali,* larva. Ventral and dorsal aspects.

tions at the surface. Leg setae all circular, elongate, barbelled, with the exception of the tarsi, which may have nude, sensory and barbelled, bayonet-type setae. Setal arrangement on legs as follows:

Coxae: I = 2; II = 1; III = 1; IV = 0;
Trochanters: I = 0; II = 0; III = 0; IV = 0;
Femurs: I = 6; II = 4; III = 3; IV = 1;
Genus: I = 4; II = 4; III = 2; IV = 2;
Tibiae: I = 5; II = 3; III = 2; IV = 2;
Tarsi: I = 13; II = 8; III = 7; IV = 7; claws well developed and curved, about 0.015 in length; pulvillae setate, only very slightly bilobed.

LARVA: (Figs. 3, 6, 7) Color as in adult, shape oval, the propodosoma with somewhat straight edges meeting at a wide angle; length, excluding gnathosoma, 0.43; width, 0.36. Gnathosome 0.09 wide at base. Chelicerae bases bearing two pairs of short, barbelled setae ventrally. Palpi well developed and three-segmented, about 0.035 long. Palpal tarsus with one short barbelled seta apically; ventrally, with two setate setae about 0.009 in length; palpal tibia about 0.015 long. Gnathosomal cuticle very lightly striate and punctate.

DORSUM: With a pair of elongate, slightly expanded, barbelled sensillae about 0.027 long preceded by a pair of tiny, expanded, barbelled presensillar setae. Dorsal setae short, expanded, and barbelled in a 4-4-2-2-2 formula. Dorsal cuticle with light striations and minute linear punctations. A distinct, narrow, peritremal line composed of two tiny rows of bead-like, irregular plates transverses the dorsum directly in front of the presensillar setae, curving ventrad to end behind coxae II.

VENTER: With two pairs of sternal setae and two pairs of genital setae, first and missing pair of genital setae reduced to a pore-like pit and a single pair of anal setae. Cuticle of venter finely striate and punctate.

LEGS: Well developed. Tarsae of legs II (Fig. 6) and III (Fig. 7) moderately enlarged and specialized to hold a pair of long, strong, equal claws ending in a short, stout hook. Cuticle very lightly striate and punctate. Setae all of a short, moderately expanded, circular, barbelled type with the exception of the tarsal setae. Arrangement of setae is as follows:

Coxae: I = 2; II = 1; III = 1;
Trochanters: I = 0; II = 0; III = 0;
Femurs: I = 6; II = 4; III = 3;
Genus: I = 4; II = 4; III = 3;
Tibiae: I = 4; II = 2; III = 2;
Tarsi: I = 11, one nude sensory, two barbelled bayonet and eight barbelled, expanded setae with normal claws; II = 6, one expanded, barbelled bayonet and five expanded barbelled setae. With a pair of strong equal, elongate claws measuring 0.03 long and 0.012 wide; III = 5, all expanded barbelled with enlarged claws as on tarsus II. All empodiae have a small, setate pulvillus which is best seen in lateral view.

DIAGNOSIS: This species is closely related to *Boydaia nigra* Fain 1955 and *Boydaia trochila* Fain 1958. It is separated from *B. trochila* by the type of setae, claws, and pulvillus of tarsus I of the adult female. *B. sturnellae* lacks the bulbed bayonets on the setae, the abruptly terminating claws and the bilobed pulvillus of *B. trochila*. The larvae of the two are much alike but may be differentiated by the configuration of the palpal tarsus, the palpal setae, and the small pulvillus of *B. sturnellae*. *B. sturnellae* may be separated from *B. nigra* by the toothed claws on leg III of *nigra* adults, lacking in

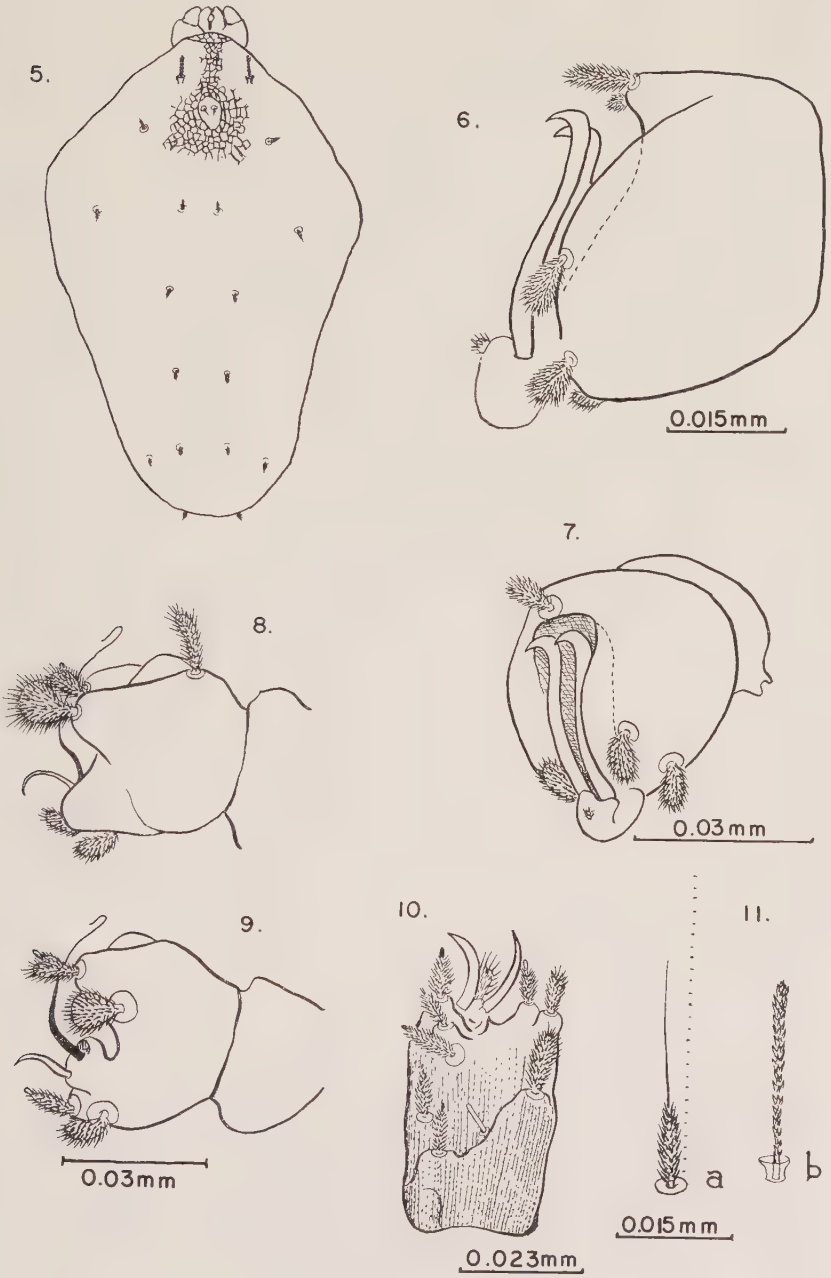

Figure 5. *Speleognathopsis sciuri,* adult. Dorsal aspect; Figure 6. *Boydaia sturnellae,* larva. Tarsus II; Figure 7. *Boydaia sturnellae,* larva. Tarsus III; Figure 8. *Boydaia quiscali,* larva. Dorsal view of tarsus II; Figure 9. *Boydaia quiscali,* larva. Ventral view of tarsus II; Figure 10. *Boydaia sturnellae,* adult. Dorsal view of tarsus I; Figure 11. *Speleognathopsis sciuri,* adult. a. Ciliated, barbelled seta. b. Sensilla.

sturnellae. In size, *B. nigra* is considerably larger than *B. sturnellae*. The specialized larval tarsi of these species may be differentiated by general configuration, type of setation, size and thickness of the claws and presence or absence of a pulvillus. *B. sturnellae* has shorter stouter claws, a small setated pulvillus, and tarsal setae which are not nearly as expanded as those of *B. nigra*.

HOLOTYPE: Female U.S.N.M. #2564 is deposited in the collection of the United States National Museum, Washington, D. C. Specimens will be filed in various museums and collections as they become available.

TYPE HOST: *Sturnella magna* (Linnaeus), the eastern meadowlark.

TYPE LOCALITY: Patuxent Research Refuge, Laurel, Maryland.

REMARKS: The species *B. nigra, B. trochila* and *B. sturnellae* are remarkably close morphologically and on this basis alone, they could, conceivably, be considered variant members of the same species. The host relationships counteract this thesis strongly. *B. nigra* has been taken only in Africa, from at least ten different genera of passeriform birds, including canaries, sparrows, and wagtails while *B. trochila* has been recovered from the genus *Colibris* (?) a genus of hummingbirds (Family Trochilidae) in South America (Fain, 1958). *B. sturnellae* has been found only in the eastern meadowlark in Texas and Maryland. The meadowlark is a member of the passeriform family Icteridae, which is peculiar to America. This bird, although extending its range into South America, is sharply demarcated from the family of hummingbirds in its feeding habits, nesting spots, and congregation areas. There appears to be little chance for the kind of intimate association between the two populations that would be necessary for the transfer of speleognathid nasal mites. Both the trochilids and the icterids have been completely separated from old-world bird genera for a very long period of time. Because of this it is quite likely that the speleognathid mites in these three groups have had sufficient time and isolation to attain the species level. The slight morphological change may be explained by the fact that the genus *Boydaia* seems to have little genetic variability in those factors governing morphology. Most of the adults in the genus are strikingly similar and it is only in the larvae that sharp morphological differences are observed. The writer believes that the speleognathids are an old group of internal parasites and that many of the species are highly host specific.

Boydaia quiscali, n. sp.

ADULT: Morphology conforms closely to other members of the genus *Boydaia* as *B. sturni* Boyd 1948 and *B. colini* Clark, 1958.

LARVA: (Figs. 4, 8, 9) Color milky white to slightly yellow; shape oval with a slight bulge in the humeral area; size, 0.4 in length by 0.3 in width, excluding gnathosoma, which is 0.07 wide at base, with three-segmented palpi measuring 0.036 in length; palpal tarsus 0.012 in length, bearing four setae, dorsally with a short setate seta, ventrally with one nude, bladelike sensory, one short barbelled and one longer barbelled seta; palpal tibia 0.018 long without setae; cheliceral bases bearing two pairs of short, barbelled setae.

DORSUM: Anteriorally, with a pair of slightly expanded, circular, barbelled sensillae 0.033 in length, preceded by a pair of short presensillar barbelled setae; dorsal setae barbelled, short and moderately expanded, arranged in a 4-4-2-2-2 formula; dorsal cuticle finely striated.

VENTER: With two pairs of short, barbelled sternal setae and three pairs of genital setae.

LEGS: Tarsus II (Figs. 8, 9) specialized and somewhat enlarged, measures (base of pulvillus to tibia) 0.03 in length and 0.036 in width; ventrally bears one flat, expanded barbelled seta, one flat expanded barbelled seta with a strong lobe and a blunt bayonet, which in side view may appear bilobed; posteriorally, with two large flat barbelled setae, one of which bears a blunt bayonet; apically, with a short, setate, slightly bilobed pulvillus and a large empodium; dorsally, with a large, elongate barbelled seta with a short blunt bayonet; apically, with one expanded, flattened barbelled seta. Claws unequal, one sinuous, curved, and extended, with a shepherd's crook, the other shorter, almost normal, curving downward, with a blunt end (Figs. 8, 9). Long claw measures about 0.030 in length. Tarsi of other legs normal. The leg setation is as follows:

Coxae: I = 2; II = 1; III = 1;
Trochanters: I = 0; II = 0; III = 0;
Femurs: I = 6; II = 4; III = 3;
Genus: I = 4; II = 4; III = 3;
Tibiae: I = 4; II = 2; III = 2;
Tarsi: I = 11; II = 6; III = 5.

DIAGNOSIS: *B. quiscali* may be distinguished from other known members of the genus *Boydaia,* particularly *B. spatulata* Fain 1956, which it closely resembles, by the characteristics and configuration of tarsus II of the larva. *B. quiscali* differs from *B. spatulata* in the position and type of the setae and the size and configuration of the claws. Tarsus II of *B. spatulata* has but one ventral seta and its subequal claw is longer and carried differently than in *B. quiscali.*

HOLOTYPE: Larva U.S.N.M. #2565 is deposited in the collection of the United States National Museum, Washington, D. C.

TYPE HOST: *Quiscalus quiscula* (Linnaeus), the common grackle.

TYPE LOCALITY: Reservoir watershed, Hanover, Pennsylvania.

REMARKS: This species is strikingly similar to *B. spatulata,* which has been found in various passeriform genera of the families Turdidae, Paridae, Nectarimidae and perhaps Motacillidae, Nectariniidae and Sylviidae, all in Africa. The long isolation of the common grackle from these groups coupled with the clear-cut differences in the configuration of tarsus II of the larvae are, it is believed, sufficient justification for the erection of *B. quiscali* as a distinct species. Here again the host relationships play a major role in differentiating the species because of the limited morphological variation inherent in this group of mites.

B. quiscali was found in but two of approximately 1,000 grackles examined from the type locality. It is notable that, although the grackle and meadowlark are both members of the American family Icteridae, they harbor clearly separate speleognathid species which are apparently completely specific. Speleognathids have not as yet been found in other icterids as the redwinged blackbird and the cowbird, hundreds of which have been autopsied and searched for nasal mites in this laboratory.

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Survival of *Taenia saginata* Eggs on Stored Hay

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The suspicion that hay contaminated with eggs of the beef tapeworm, *Taenia saginata*, might have been a source of infection in certain outbreaks of *Cysticercus bovis* infection recently investigated by disease-eradication personnel was brought to our attention several months ago. Accordingly, we carried out an experiment in an effort to determine how long hay known to have been so contaminated need be stored to destroy the viability of the eggs.

Available chains of proglottids and free eggs of this tapeworm* were divided into aliquots. The control aliquot in saline solution was immediately placed in a refrigerator. On the same day, each of the remaining aliquots was placed on alfalfa hay in a separate glass tube about 6 inches in length and 1¼ inches in diameter. In the middle 2 inches of the tube were placed a layer of crumbled alfalfa leaves, an aliquot, and another leafy layer; from here to each end, the tube was lightly plugged with a layer of stemmy hay and a leafy layer; each end of the tube was covered with two-ply cheesecloth. Each aliquot consisted of (1) six proglottids which appeared to be definitely gravid, (2) six whose ripeness was doubtful, and (3) about 180,000 free brown-shelled eggs contained in about 5 ml. of saline solution. The tubes were kept in a horizontal position at room temperature until the next day. Then the control aliquot was removed from the refrigerator and transferred to a gelatin capsule which was administered to a calf and the tubes were inserted horizontally into a bale of alfalfa in a hay loft. They were inserted along the axis of the bale.

When a tube was removed from the bale, its contents and a small volume of water in which the emptied tube had been thoroughly washed to remove any adherent eggs were admixed with calf meal. Each of the hungry calves to which this mixture was offered quickly and completely consumed it, as well as additional calf meal moistened with washings from the tray in which the mixture had been placed. The calves used in the experiment were reared and kept at the Agricultural Research Center, Beltsville, Maryland. Extraneous infection with *C. bovis* has not been detected in any of a large number of cattle that had been kept on the same premises under the same conditions.

*Obtained through the cooperation of Rex W. Allen, ADP, ARS, State College, New Mexico.

Calf 1, which received the control aliquot and was slaughtered 63 days later, was found to be heavily infected with cysts of *C. bovis*. When the tissue was finely sliced with a knife, the following numbers of cysts of various sizes were found in weighed amounts of the musculature from various sites: Heart—251 in 110 grams; masseter—90 in 85 grams; diaphragm—20 in 45 grams; hind legs—28 in 270 grams; shoulders—7 in 75 grams; neck—18 in 110 grams; intercostal—2 in 45 grams; abdomen—15 in 75 grams. Five cysts were found in the tongue, which was completely examined; 12, in the nearby buccal tissues; and 7, in the muscular layer of the esophagus.

Only 1 cyst, located in the cheek, was found in calf 2, which received an aliquot stored in the hay for 22 days (range in outdoor temp., 34 to 87° F.) and was slaughtered 77 days later. None was found in calf 3, which received an aliquot stored in the hay for 71 days (range in outdoor temp., 11 to 87° F.) and was slaughtered 70 days later. The heart, including its external and internal surfaces, diaphragm, tongue, masseter muscles, and the muscular layer of the esophagus of each of these calves were completely, or nearly completely, examined; the sample examined from each of the other aforementioned sites was considerably larger than the corresponding sample taken from calf 1, and numerous other incisions were made in the carcass for inspection of the cut surfaces. In each case the examination was much more extensive than the initial procedures of inspection proscribed by Federal meat-inspection regulations for determining whether carcasses of cattle are free from or infected with *C. bovis*.

From these findings, we conclude that a small percentage of *T. saginata* eggs deposited on hay kept under the conditions here described remained viable for about 3 weeks; however, none remained viable for as long as 10 weeks. Dessication was probably the factor mainly responsible for their loss of viability. Silverman (1956. Trans. Roy. Soc. Med. and Hyg., 50:8) found that eggs of this tapeworm did not survive longer than 14 days in the absence of surface moisture, irrespective of relative humidity.

The carcass of calf 1 contained a large number of small and very small "cysts," which were especially conspicuous in the heart, as well as many cysts approximately normal in size for their age (9 weeks). Each of the larger cysts dissected contained a well-developed *C. bovis* larva; larvae examined on the day the calf was slaughtered were alive. Recognizable cestode tissue was not obtained from the small cysts.

However, on histologic examination it was observed by Dr. W. T. Shalkop (ADP) that many of the small cysts had been replaced by lymphoid cells along with a few neutrophiles and an occasional eosinophile. These small reactive foci, some of which were centered around an unidentifiable foreign body, resembled the host-tissue reaction that had developed around recognizable *C. bovis*. Therefore, it seems reasonable to assume that they were provoked by *T. saginata* larvae that had died in early stages of development.

Fiftieth Anniversary
Helminthological Society of Washington

The fiftieth anniversary of the Helminthological Society of Washington falls on October 8, 1960. The society plans to observe the occasion by holding a special all-day meeting, followed by a banquet, and to issue a commemorative number of the Proceedings. Members are urged to make plans now to attend this special meeting and banquet. A letter giving details will be sent to members.